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BULLETIN OF THE TORREY BOTANICAL CLUB
VOL. 77, NO. 5, PP. 323–339 SEPTEMBER, 1950

THE GAMETOPHYTE OF THE GLEICHENIACEAE[1, 2]

ALMA G. STOKEY

In recent years the family Gleicheniaceae has received considerable attention in taxonomic works. Before the middle of the 19th century six genera had been recognized in this well-defined family, although Hooker, neither in 1846 nor later, accepted more than two—*Gleichenia* Sm. which he divided into four sections, and the monotypic *Platyzoma* R. Br. By the latter part of the 19th century most authors accepted only one or two genera. Christ (1897) limited the family to the genus *Gleichenia* and divided it into four sub-genera. Diels (1902) recognized two genera—the monotypic *Stromatopteris* Mett. and *Gleichenia* the latter divided into three sub-genera with four sections in the Mertensia group. Christensen (1906) recognized the same two genera, dividing *Gleichenia* into the sub-genera Dicranopteris, Eugleichenia and Platyzoma. Underwood (1907) accepted *Gleichenia* and *Stromatopteris*, reviving also *Dicranopteris* Bernh. and *Platyzoma* as genera. Christensen (1938) accepted these four and revived the Presl genus *Sticherus*. Ching (1940) and Copeland (1941), both of whom recognized these five genera, revived, independently, another Presl genus, *Hicriopteris*, but Ching also segregated from *Dicranopteris* a seventh, *Gleicheniella* Ching, to accommodate the solenostelic *D. pectinata*.

The gametophyte has received compartively little attention, probably because the members of this tropical family are rarely found in cultivation. There are no accounts of the gametophyte except those based on the investigations of Rauwenhoff (1879, 1890) and Campbell (1908). Diels (1902) in his discussion of the Gleicheniaceae disposed of the gametophyte in less than three lines, as, from Rauwenhoff's accounts he considered it too like the

[1] The author wishes to express her thanks to the members of the staff of the Botanical Gardens at Buitenzorg and Tjibodas, Java, for providing the excellent facilities for collection and work which she enjoyed in 1931 and 1937, and to Dr. O. Posthumus for the determination of the species of ferns collected in Java; to Professor S. Hibino, University of Taihoku, for assistance in collecting ferns in Formosa and to the late Professor Kudo for their identification; to the late Dr. L. A. Boodle through whose kindness spores were sent from the Royal Botanic Gardens, Kew; to Dr. M. A. Chrysler for a collection from Puerto Rico; to Mr. C. Bernard Lewis, Curator of the Science Museum, Institute of Jamaica, for his kindness in arranging facilities for collecting ferns, to Mrs. A. Vonder Porten of the Science Museum for collections of spores in Jamaica, and to the late Mr. C. A. Weatherby for the determination of Jamaican species; and to Dr. Lenette R. Atkinson for assistance with cultures and preparation of slides. The work was carried out at the Treub Laboratorium, Buitenzorg, Java; the Marine Biological Laboratory, Woods Hole, Mass.; and at Mount Holyoke College.

[2] Illustrations published with the assistance of the Lucien M. Underwood Memorial Fund.

[THE BULLETIN for July–August (77: 245–322) was issued July 18, 1950]

Polypodiaceae to merit special attention. Rauwenhoff (1879) described the structure of the spore and especially the spore coat of several species. In 1890 he published his observations on the gametophytes of five species which he had maintained in culture, intermittently, over a period of ten years: *G. rupestris, G. circinnata, G. dicarpa, G. hecistophylla* (*G. dicarpa* R. Br.) and *G. flabellata* (*Sticherus flabellatus* (R. Br.) St. John). Campbell described the habit and structure of the mature gametophyte of four species collected in the field: *G. polypodioides* (L.) Sm., *G. laevigata* (Willd.) Hook. (*S. laevigatus* (Willd.) Presl), *G. dichotoma* (Thunb.) Willd. (*D. linearis* (Burm.) Underw.), and G. *pectinata* Willd. (*D. pectinata* (Willd.) Underw. or *Gleicheniella pectinata* (Willd.) Ching).

The present investigation is based on cultures made from spores obtained from various sources, most of them collected in the field by the author. The species are listed below with sources and length of time in culture:

Dicranopteris pectinata (Willd.) Underw.	2 yrs.; 18 mon.	Jamaica
Gleichenia circinnata Sw. var. Cult.	9 mon.	Kew
Gleichenia rupestris R. Br. var. Cult.	5½ mon.	Kew
Gleichenia vulcanica Bl.	4 yrs. 5 mon.	Java
Hicriopteris Bancroftii (Hk.) Ching	10 mon.	Jamaica
Hicriopteris glauca (Thunb.) Ching	17 mon.	Formosa
Hicriopteris volubilis (Jungh.) Ching	17 mon.	Java
Sticherus bifidus (Willd.) Ching	13 mon.	Puerto Rico
	18 mon.	Jamaica
Sticherus palmatus (Schaff.) Copel.	7 mon.; 1 yr.	Jamaica
Sticherus vestitus (Bl.) Ching	13 mon.	Java

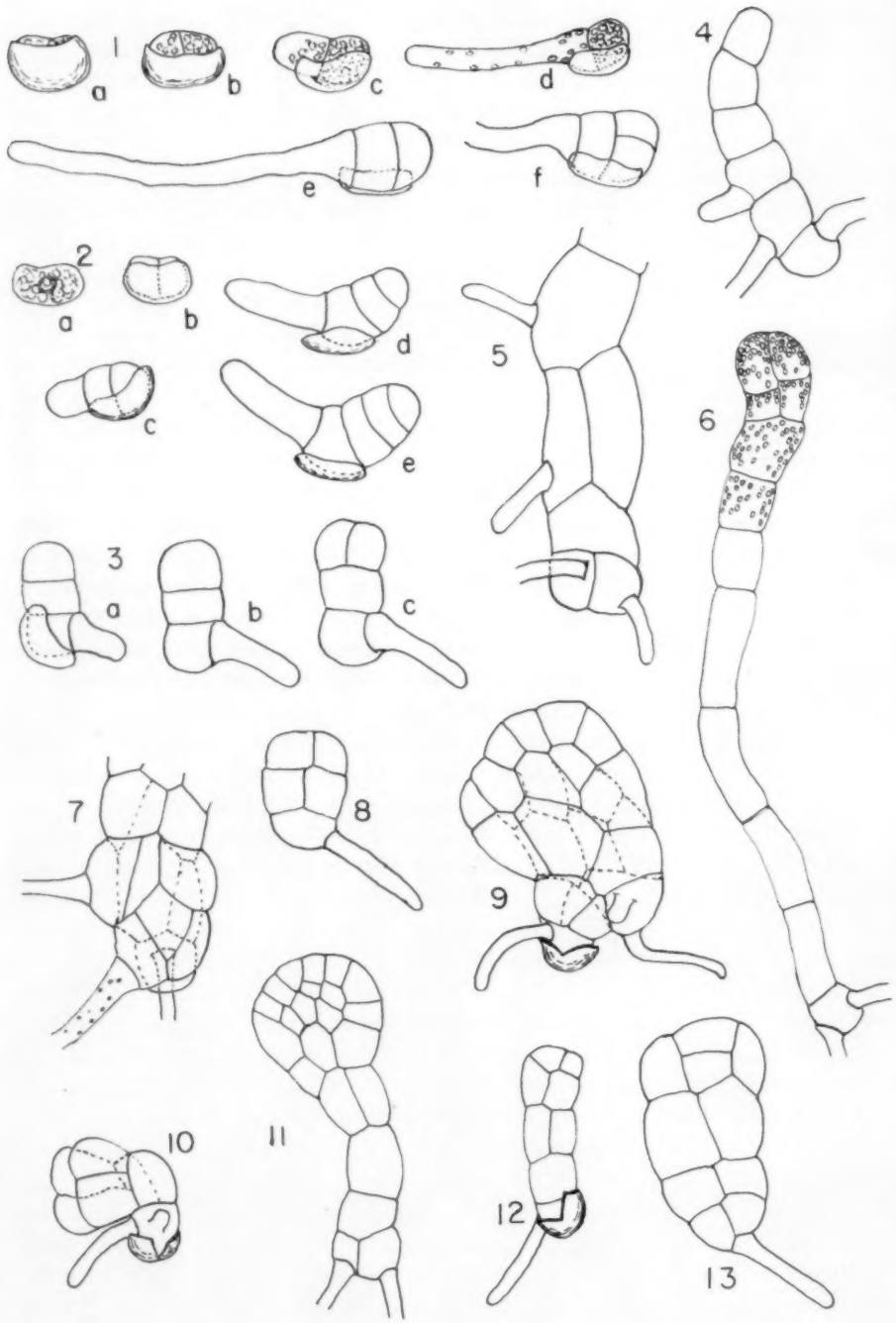
Methods. The spores were planted as soon as possible after collections were made. Some spores were viable for five or six months but in general the spores of this family do not retain their viability for long periods. In much of the material a larger proportion of the spores was found to be non-viable than is usually the case with fern spores. The early stages of germination of the spore and of the young gametophyte were studied from cultures on distilled water, but in the case of *S. bifidus* and *S. palmatus* several series of cultures were made on plates of silica gel containing a small amount of nutrient salts, according to the method described by Hanks and Weintreub (1936). This medium has the advantage over agar that common molds do not develop on the silica gel and this makes it possible to examine the cultures without a cover under a high power lens, but it has the disadvantage that the silica gel dries out more rapidly and breaks down. Because of the slow germination of spores and slow growth of the young gametophytes in this family, only early stages were obtained on silica gel.

Fresh material was used for germination of spores, early stages of the gametophyte, mature habit, distribution of rhizoids, hairs and sex organs.

The gametophytes to be sectioned were killed and fixed in various solutions: medium chromo-acetic acid, Nawaschin's fluid (Craf), formalin acetic alcohol and formalin propionic alcohol. Sections were cut usually at 10 micra and stained with Flemming's triple stain, safranin and fast green, and Haidenhain's iron alum haematoxylin.

Germination of spore and vegetative structures. This family can hardly be said to have a characteristic type of germination and early development of the gametophyte. Probably in no family of ferns does greater plasticity exist in the early stages of the gametophyte. The spores of the family are of the radiate tetrahedral type, as in *Gleichenia* and *Hicriopteris,* or of the bilateral type, as in *Sticherus* and *D. pectinata.* The spores are smaller than in most ferns as is indicated by the following measurements (average of five): *S. bifidus* and *S. palmatus* 38×20 μ; *S. vestitus,* 40×23 μ; *D. pectinata,* 38×17 μ; *H. glauca,* 27 μ; *H. volubile* and *H. Bancroftii,* 31 μ; *G. vulcanica,* 45 μ. The spores are more or less homogeneous in content or with a faintly granular appearance or with a suggestion of oil drops. The coat is thin and smooth; the color of the spore is white or of a yellowish cast. When germination is near, granules and the nucleus can usually be seen very plainly (fig. 2 *a*). In some cases there appears to be a division of the spore contents before the cracking of the spore coat. Germination is relatively slow in this family, and the spore coat does not crack until two or three weeks after the planting of the spores. In these species it was not observed until 16 days, and in most cases not until 18–20 days, or even later.

The germination and early stages were followed more closely in *S. bifidus* and *S. palmatus* than in the other species. In these the first wall is perpendicular to the long axis of the spore, and separates the first rhizoid from the first prothallial cell (fig. 1 *b, c*). There is considerable enlargement before another division occurs in the prothallial cell. In the silica gel cultures germination often resulted in a short filament (figs. 2, 4) and this is often the case on water and on peat, but the early initiation of a plate by the longitudinal division of the second prothallial cell occurs frequently (figs. 1 *f,* 8, 13). Less frequently a mass forms at an early age (figs. 9, 10). There does not seem to be the same strong tendency towards plate or mass formation that is found in the Marattiaceae and Osmundaceae. However, plates similar to those of the Osmundaceae may be found (fig. 8). The cell arrangements in the Gleicheniaceae are in general much less regular and show greater variation than those in the Osmundaceae. When germination takes place on peat there are more cases of irregular germination as a mass. A filament usually consists of a few relatively short cells (fig. 4), less commonly of elongated cells (fig. 6). Even in filamentous prothalli the base may be divided longitudinally and there may be intercalary divisions (figs. 5, 11). When spores are crowded on the substratum they usually germinate as filaments, which, when space permits, pass on into a plate or mass; a filament or plate may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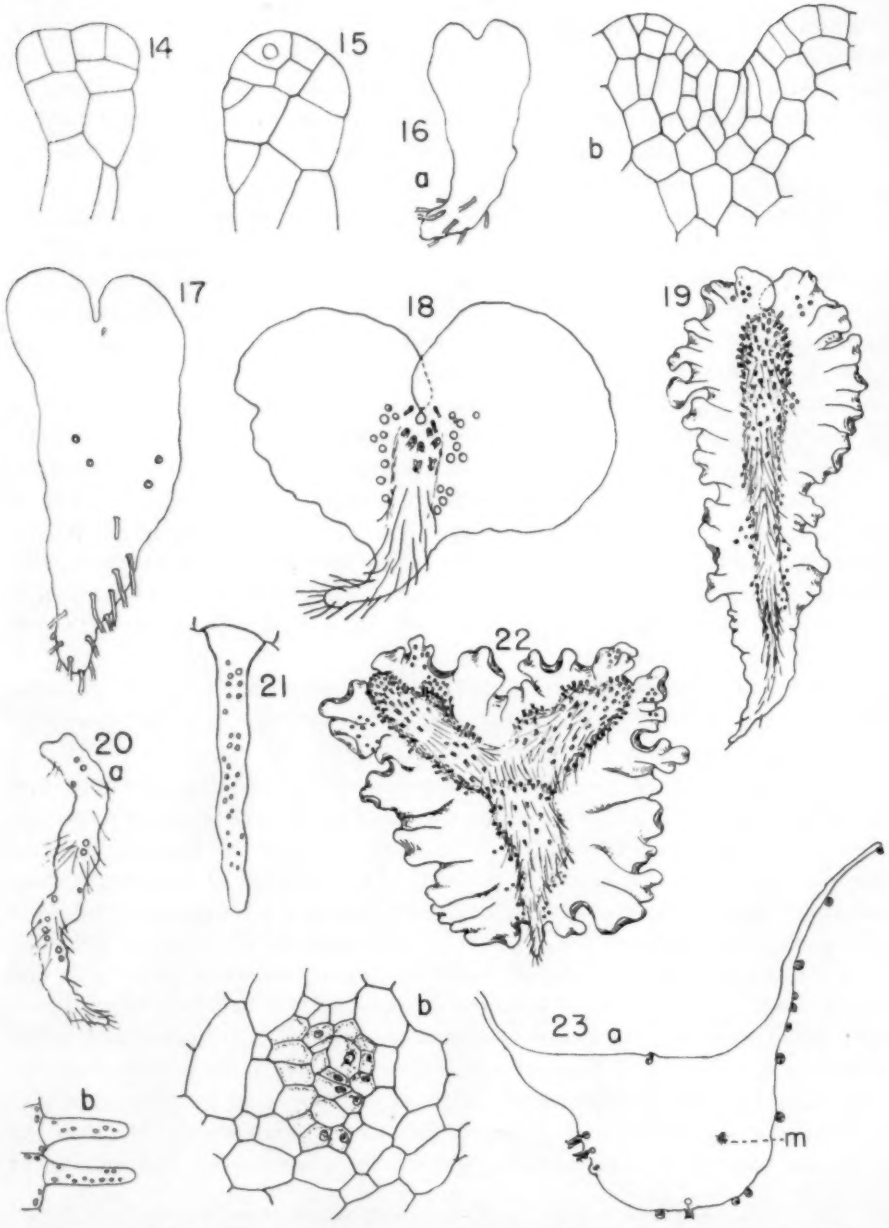
expand into a mass and then revert into a plate. The same plasticity and variability in the early stages was found in all four genera. Rauwenhoff found similar variation in his four species of *Gleichenia* and in *S. flabellatus*. Examination of gametophytes which have grown to a large size and formed a heavy midrib, usually reveals a massive base; even in relatively young gametophytes the base is often thick and club-shaped (figs. 7, 16, 17).

The broadening of the thallus is more gradual than in many ferns. The young gametophytes are usually of a spatulate form, developing rather slowly into an elongate cordate form (figs. 16 *a*, 17). The broadening of a filamentous gametophyte begins ordinarily by a longitudinal division in the terminal cell (figs. 3, 6, 12, 14); after several divisions have occurred in one or both of these cells at the tip, an apical cell can usually be identified (figs. 11, 15, 16 *b*). The wedge-shaped apical cell functions for a period but is ultimately replaced by a group of meristematic cells as in most fern gametophytes. There are many irregularities and variations especially in crowded cultures, but ameristic and persistently filamentous prothalli are not as common as in the more advanced ferns. A slender elongated plate is a common type in crowded cultures (fig. 20 *a*). In favorable conditions the broadening proceeds more rapidly and consistently, but there is usually a longer tapering portion at the base than is usually associated with the cordate type (figs. 18, 19).

The mature gametophyte is notable for the heavy midrib and the uplifted wings with a more or less irregular margin. The midrib projects strongly on the ventral side and on the dorsal side has a gutter-like aspect. As the thallus elongates the wings take on a ruffled appearance (fig. 19). The gametophytes in culture are of a brighter green than those of the Marattiaceae and Osmundaceae, and although the midrib is as heavy they do not appear to be as massive. The early thickening of the midrib is indicated by the longitudinal sections (figs. 37, 64–66, 75); it commonly attains a thickness of at least 10–12 cells, but in gametophytes a year old it may be 15–18 cells thick (fig. 23 *a*). The midrib of *G. vulcanica* was not found to exceed 10–11 cells and never reached the thickness attained by species of *Sticherus*, *Hicriopteris* and *Dicranopteris*. The heavy midrib and ruffled wings give a distinctive aspect to the prothallus suggestive of a liverwort, but none in these cultures ever suggested the leaf-like habit such as is mentioned by Campbell who compared the prothallus of *S. laevigatus* to a *Fossombronia*. He said the gametophytes of *G. polypodioides* showed the least lobing and ruffling of

FIGS. 1–13. Germination of the spore and early stages of the gametophyte. FIG. 1 *a–f*. Series from 20–35 days after planting. FIG. 2. *a–e*. Series 21–35 days. FIG. 3. *a–c*. Series 25–30 days. FIG. 4. Filament 32 days. FIGS. 5, 7. Base of young gametophyte. FIG. 6. Filament 39 days. FIG. 8. Young plate. FIGS. 9, 10. Young prothalli with division in three planes. FIG. 11. Thallus with apical cell. FIGS. 12, 13. Early stages of thallus. FIGS. 1–7. *Sticherus bifidus*. FIG. 8. *S. vestitus*. FIGS. 9, 10. *Hicriopteris volubilis*. FIGS. 11–13. *Gleichenia vulcanica*.



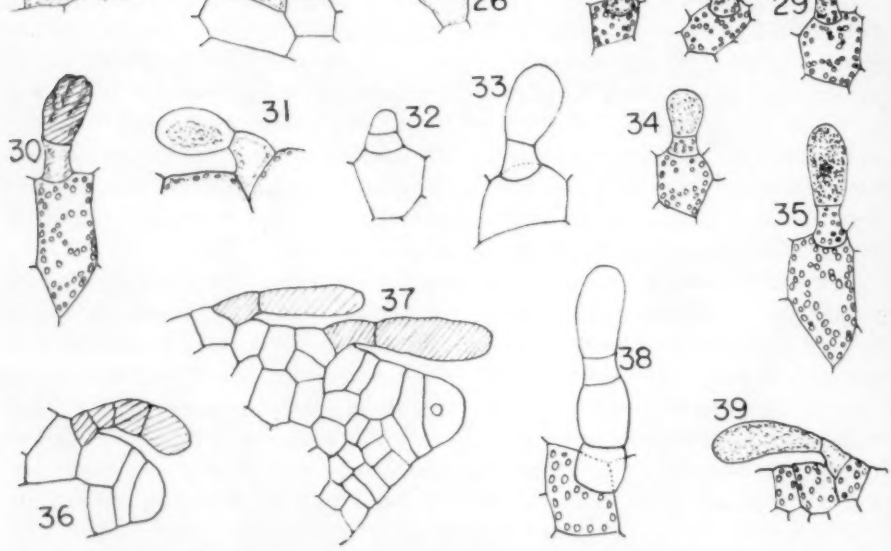
Figs. 14–23. Stages in development of the gametophyte. Figs. 14, 15. Tip region of young gametophyte. Fig. 16 *a*, *b*. Young gametophyte; detail of apex. Fig. 17. Gametophyte 60 days old with four antheridia and one hair. Fig. 18. Mature gametophyte; 75 days old. Fig. 19. Gametophyte nine months old. Fig. 20 *a*. Young male gametophyte; *b*, rhizoids from near tip of *a*. Fig. 21. Rhizoid with chloroplasts from thallus 8 months old. Fig. 22. Gametophyte one year old. Fig. 23 *a*. Cross section of thallus 17–18 cells thick with meristematic strand *m*; *b*, meristematic strand. Figs. 14–19. *Sticherus bifidus*. Fig. 20. *S. vestitus*. Fig. 21. *Hicriopteris volubilis*. Figs. 22, 23. *H. glauca*.

his four species, but his figures indicate that his collections of *G. polypodioides* (his smallest collection) were younger than those of his other three species. Although his older prothalli of *D. linearis* are almost as lobed and ruffled as those of *S. laevigatus*, the younger stages in his figures are similar to those of *G. polypodioides*, or to those of *S. bifidus* shown in the present paper (fig. 18). Rauwenhoff's cultures were not carried to the age at which the ribbon-like aspect is attained. Branching of the midrib and of the thallus with the development of two apical regions may be seen in large vigorous gametophytes. Several cases were found in *H. glauca* when 11–12 months old (fig. 22) and in *G. vulcanica* when several years old. Campbell reported forking in large gametophytes collected in the field.

The first rhizoids are pale with a good complement of chloroplasts which persist for a surprisingly long time, but disappear as the rhizoid attains its full growth and turns brown. Rhizoids which develop on gametophytes many months old may show chloroplasts when young as in fig. 20 *b*, which shows two young rhizoids from near the tips of fig. 20 *a*, and also in fig. 21, a rhizoid from a thallus eight months old. The rhizoids in all these species were stout, abundant and of various shades of brown, chiefly reddish brown. Their conspicuous development was noted also by Rauwenhoff and Campbell, and is apparently a characteristic feature of this family.

Characteristic hairs appear at some time in the development of the gametophyte of many and perhaps all species of the Gleicheniaceae. The hair arises from a special initial cell. This is a wedge-shaped cell cut off obliquely from the anterior face of a cell on the midrib or wings not far from the archegonial region (figs. 24–26, 36, 37). The origin of the hair is best seen in section but its position is readily seen in fresh material (figs. 27–30, 32–35). The hair ordinarily consists of two cells, a short stalk-like cell and a larger terminal cell which is pale when young but darkens with age, and in some cases, at least, contains tannin. Three- or four-celled hairs occur occasionally but are not common (figs. 36, 38). The type of hair found in the Gleicheniaceae differs both in origin and appearance from those of *Notholaena* (Giauque, 1949), *Dryopteris*, *Anemia* or *Lygodium*. There is no indication of any substance secreted externally. The hairs are ordinarily found only on the dorsal or ventral surfaces, but they may occasionally be found on the margin (figs. 31, 39).

In *S. bifidus* the hairs made their appearance on the prothallus about two months after the sowing of the spores at the time when the first antheridia were developing (fig. 17). Three hairs are shown in fig. 18 on a gametophyte 75 days old bearing antheridia and archegonia. The hairs increased in number for a time and then their formation ceased; none were found on prothalli eight or nine months old. In one rather weak set of cultures of *S. palmatus* no hairs were ever seen although the cultures lived to be seven months old and bore both antheridia and archegonia, but in another and



FIGS. 24–39. Hairs. FIGS. 24–26. Stages in development of hair; arrow points towards apex of thallus. FIGS. 27–30. Stages in development of hair, ventral surface. FIGS. 31, 39. Marginal hairs. FIGS. 32, 33. Hairs from dorsal surface. FIG. 36. Section through apex with four-celled hair. FIG. 37. Section through apex. FIG. 38. Four-celled hair. FIGS. 24–30, *Sticherus bifidus*. FIGS. 31–33. *Hiciopteris glauca*. FIG. 34. *H. volubilis*. FIG. 35. *H. Bancroftii*. FIG. 36. *Dicranopteris pectinata*. FIGS. 37–39. *Gleichenia vulcanica*.

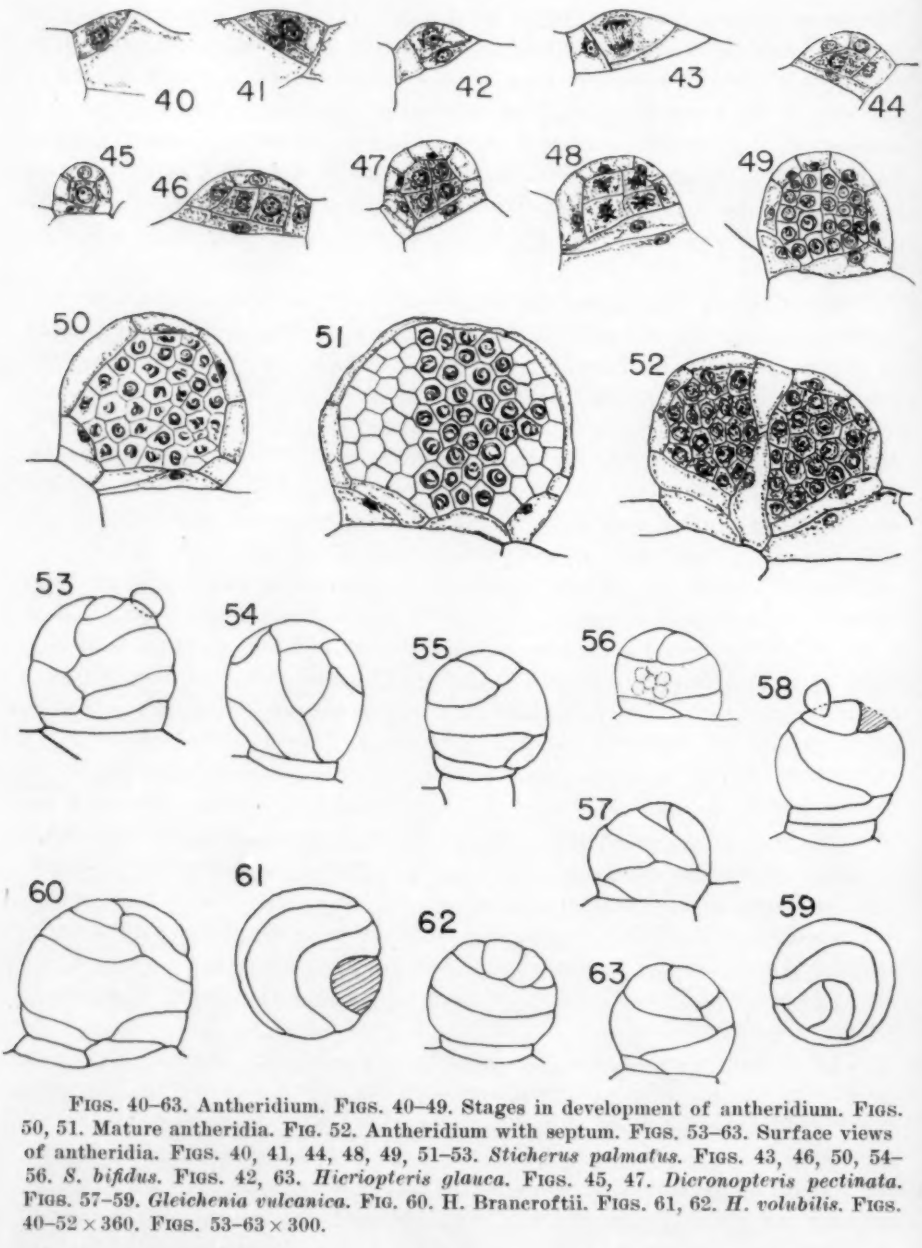
stronger set of cultures the gametophytes had about as many hairs as those of *S. bifidus*. *H. glauca* began to bear hairs when three months old, just as antheridia began to appear, and the development of hairs continued for some months, but they ceased to form before the prothalli were a year old. As hairs grow old they shrivel and may become hidden by rhizoids, so that their presence may not be noted unless they continue to form throughout the life of the gametophyte. In the case of *G. vulcanica* no hairs were found on gametophytes less than six months old and then they began to appear. They varied in number from time to time, but as is suggested by fig. 37, they were usually abundant and continued to develop until the death of the cultures when over four years old. All species in this investigation except *G. rupestris* and *G. circinnata* were found to bear hairs, but their failure to appear in these two species may have occurred because the cultures did not live long enough or were not sufficiently vigorous. These two species may have a belated development of hairs like *G. vulcanica*, rather than the early habit of *Sticherus* or *Hiciopteris*. Rauwenhoff did not observe them in his cultures and merely says that no marginal hairs like those of *Anemia* and

Platycerium were found. Four of his species were of the genus *Gleichenia* in which the hairs appear late if the condition in *G. vulcanica* is typical, and none of his cultures were long-lived. His cultures of *S. flabellatus* while short-lived did reach the age of bearing antheridia and archegonia, but this species may be variable in respect to hairs like *S. palmatus*. Campbell found hairs on the gametophytes in his collections in the field and noted that the hairs were borne in the region of the archegonia. His figure 107 of a section of *G. polypodioides* shows a characteristic two-celled hair with a wedge-shaped base.

Reproductive structures. The gametophytes of all species of the Gleicheniaceae are monoecious so far as has been observed. The earliest appearance of antheridia noted in these cultures was in *S. bifidus* seven weeks after the sowing of the spores; archegonia followed in about three weeks. In *G. circinnata* the first antheridia were seen in cultures 12 weeks old, with archegonia appearing three weeks later. Gametophytes of *H. glauca* bore antheridia when three months old, but archegonia were slow in appearing. At six months the gametophytes had only 12–13 archegonia but when a year old they had more than one hundred archegonia.

The antheridia are usually produced in great abundance and are conspicuous on the ventral side of the midrib and wings but are rare on marginal cells. They develop occasionally but less abundantly on the dorsal side in all species. Although antheridia continue to appear after archegonia have begun to develop, there is usually a reduction in their number; gametophytes which are actively bearing archegonia have relatively few young antheridia. At any time a change in the environment may cause an active production of antheridia. On a few old gametophytes of *G. vulcanica* antheridia were found interpolated among the archegonia. In crowded regions of a culture slender elongated gametophytes with a shallow notch (fig. 20) may be found bearing antheridia in varying numbers, even as high as 40–50. Ameristic irregular prothallia may produce large numbers of antheridia, but this is not a common type in this family. No filamentous gametophytes bearing antheridia, such as are common in some of the higher families of ferns, were observed.

The antheridium begins its development with an initial cell which usually is cut off obliquely from a superficial cell of the thallus (fig. 40). A series of three or more oblique intersecting walls forms the cells which constitute the base and part of the lateral wall of the antheridium (figs. 41, 42). A wall then cuts off a cap which leaves a central cell as a primary spermatogenous cell (figs. 43, 44). Fig. 44 suggests a potentially large antheridium such as may be found on the wings of a vigorous thallus; mature antheridia on this thallus had 50-55 spermatocytes in a section. But fig. 45 which is in almost the same stage, is probably an early stage of a potentially small antheridium, as it is from a thallus with small crowded antheridia



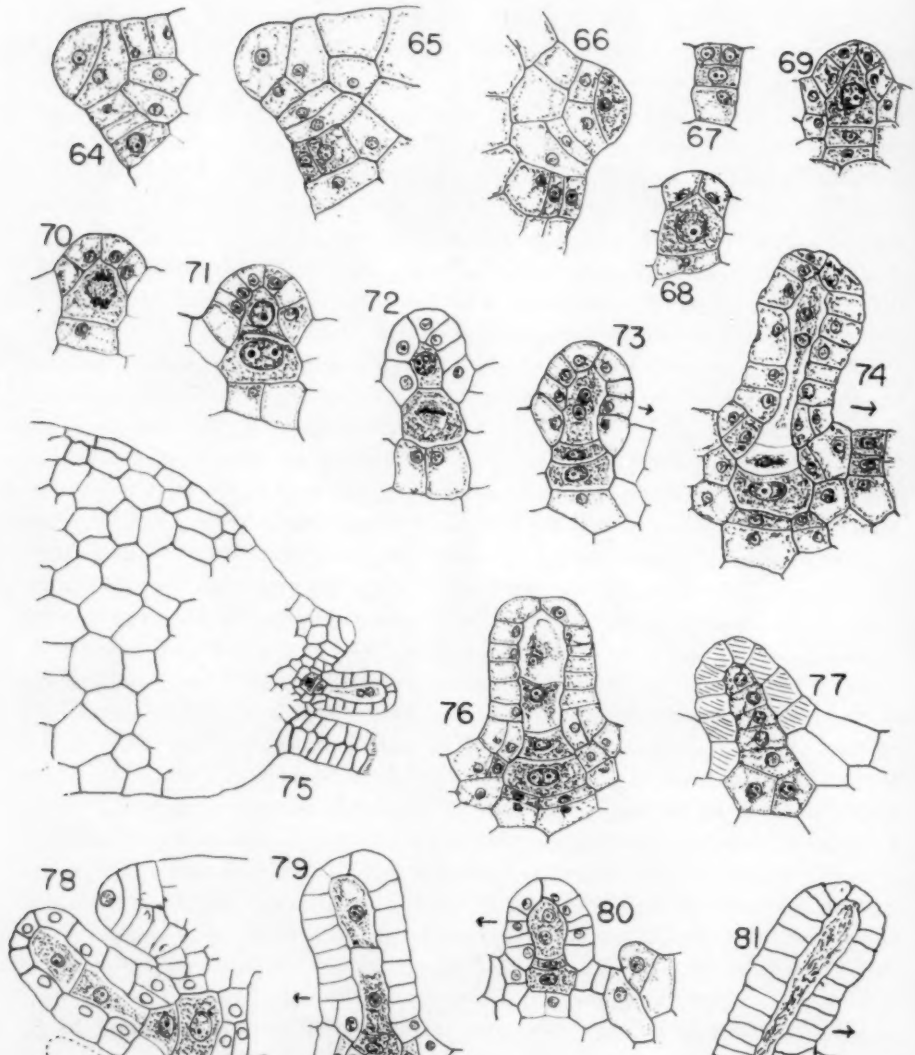
FIGS. 40–63. Antheridium. FIGS. 40–49. Stages in development of antheridium. FIGS. 50, 51. Mature antheridia. FIG. 52. Antheridium with septum. FIGS. 53–63. Surface views of antheridia. FIGS. 40, 41, 44, 48, 49, 51–53. *Sticherus palmatus*. FIGS. 43, 46, 50, 54–56. *S. bifidus*. FIGS. 42, 63. *Hiriopteris glauca*. FIGS. 45, 47. *Dicronopteris pectinata*. FIGS. 57–59. *Gleichenia vulcanica*. FIG. 60. H. Brancroftii. FIGS. 61, 62. *H. volubilis*. FIGS. 40–52 × 360. FIGS. 53–63 × 300.

with only 18–30 spermatocytes in a section. As the antheridium wall and spermatogenous tissue develop (figs. 46–49), the cap cell undergoes at least one division, but more commonly several, resulting in a small opercular cell

which varies in shape but is usually more or less oval and more often on one side than at the apex of the antheridium. The last formed cells of the antheridium may arise in such a way that the appearance from above is like that of fig. 61 and from the side like that of fig. 62; or, as is more common, the last cell walls arise as in fig. 59 and then the side views are more irregular (figs. 53, 54, 60). While the spermatogenous tissue is increasing in amount the cells forming the wall of the antheridium undergo a twisting which makes for great irregularity and variety (figs. 53–55, 57, 60, 63). Although the base is usually stalk-like many antheridia do not show a definite stalk but merely a broad base. The opercular cell is cast off at maturity and may persist intact for several hours (figs. 53, 58).

As is usual in ferns there is a wide range in size between the smallest and the largest antheridia in any species, but it is particularly striking in the Gleicheniaceae because of the great size which antheridia may attain (figs. 50, 51). Some antheridia may have no more than six cells in the wall and suggest the antheridium of *Cyathea,* while others may have 10–12 cells. The number of spermatocytes visible in a section may range from 17 to 90. This implies a possible sperm output of over 500, theoretically 512. In general the greatest differences may be related to the age and vigor of the prothallus, with small antheridia borne on young gametophytes, or on regenerated branches, or crowded on old gametophytes which are not growing vigorously. Fig. 56 shows a small antheridium from a slender male gametophyte of *S. bifidus,* a species which produces notably large antheridia with 50–85 sperms visible in a section. It should be noted, however, that none of the antheridia found on the prothalli of the three species of *Gleichenia* were nearly as large as the largest of those of *Sticherus, Hicriopteris* and *Dicranopteris.* Rauwenhoff who made a detailed study of the antheridium describes it as a relatively simple structure with few cells in the wall and a low sperm content, in general similar to that of the Polypodiaceae. However, it should be remembered that he worked with gametophytes which were neither very old nor very large, and that most of his work was on the genus *Gleichenia* although many of his figures are from *S. flabellatus.* Campbell found very large antheridia on the gametophytes which he collected in the field. In *D. pectinata* there were more than 50 spermatocytes in a section of an antheridium and he considered it probable that there were more than 12 cells in the wall layer. In *S. laevigatus* he found the antheridium even larger; his figure shows over 90 spermatocytes in a section. He found the antheridium of *G. polypodioides* more like those described by Rauwenhoff, although he found more spermatocytes and more cells in the wall. A peculiar feature seen in sections of several of the antheridia of *S. palmatus* was the development of sterile tissue which made a septum through the spermatogenus tissue (fig. 52).

The archegonium develops from an initial which is usually close to the



FIGS. 64–81. Archegonium. FIGS. 64–74. Stages in development of archegonium. FIG. 75. l.s. of apex. FIGS. 76,78,79. Archegonia with two neck canal cells. FIG. 77. Anomalous archegonium. FIG. 80. Archegonium and young hair. FIG. 81. Old archegonium. FIGS. 64–76. *Sticherus bifidus*. FIG. 77. *S. palmatus*. FIG. 78. *Dicranopteris pectinata*. FIGS. 79-81. *Gleichenia vulcanica*.

apical cell in a region which is most often four cells thick (fig. 64). The first division cuts off the primary neck cell (fig. 65), the second cuts off the basal cell making the characteristic tier of three cells (figs. 66, 74). The primary neck cell soon divides into the initials of the four tiers of neck cells (fig. 67). They are soon pushed out by the enlargement of the cell beneath and as the enlargement continues the neck cells lengthen and divide (figs. 68, 69). The primary cell of the axial row is then divided by a wall into a central cell and the primary neck canal cell (figs. 70, 71). Shortly afterwards the ventral canal cell is cut off from the egg (fig. 72). Occasionally a division of the basal cell occurs in one of these early stages (figs. 69, 71, 72). The division of the nucleus of the primary neck canal cell completes the axial row as it is found in most archegonia (figs. 73, 74, 80). However, the formation of a wall which is usually very delicate between the nuclei of the neck canal is not uncommon (figs. 76, 78, 79). It was found in all four genera. There were other cases in which there was a definite break in the protoplast of the neck canal with a separation of the two nuclei but no wall could be detected. Campbell said the presence of a wall was the usual thing in three of his species but not in *G. polypodioides*. No case was found of the increase of the neck canal nuclei to four as was found in *Lygodium palmatum* (Rogers, 1927) and in the Cyatheaceae and Dicksoniaceae (Stokey, 1930). There is often seen at some stage in the development of the archegonium a modification of the walls of the neck cells, presumably cutinization as indicated by certain stains especially Flemming's triple stain, and a change in the staining reaction of the contents, perhaps associated with the disappearance of the chloroplasts. At the time of the completion of the axial row there are usually only four cells in each tier of the neck, but by the time the neck is ready to open the number has increased to seven, eight or nine. It is not unusual to find the long necks projecting beyond the apical cell (figs. 75, 78) even while the apical region is still forming young archegonia. Sections adjoining that shown in fig. 75 had young archegonia in the stage of the tier of three. The neck is approximately straight or slightly curved forward at the base when it is ready to open. Owing to the great thickness of the thallus and the slope of the ventral cushion in the region where archegonia are maturing, the archegonia which project towards the apex may be almost parallel to the substratum at the time when they open. The growth of the midrib may be such that the necks of many older archegonia will be found projecting laterally from the midrib. On the older prothalli of *G. vulcanica* there were archegonia with unusually long necks which had as many as 12–14 cells in a tier, and in this type the forward tilt at the base was pronounced (fig. 81). Campbell noted this type in *G. polypodioides*. Although a division of the basal cell may occur at an early stage of the archegonium the formation of a nutritive jacket layer surrounding the venter does not

take place until after the last divisions have occurred in the axial row and the archegonium is in its final stage of development (figs. 74, 76, 79).

In *Sticherus palmatus* an archegonium was seen in which a definite tissue had developed instead of the axial row in the venter and neck (fig. 77), but this gave no suggestion of sporophytic tissue. The thallus on which it grew had a younger archegonium which also suggested anomalous development, but also a young stage of an apparently normal archegonium with the axial row almost complete. No cases of apogamy were found in any species investigated, and no suggestion of it except for a strand of small meristematic cells running through a large thallus of *Hicriopteris volubilis* (figs. 23 *a, b*). This strand extended through 16 sections beginning and ending blindly. At the stage at which the thallus was killed there was no indication of sporophytic character in the cells of the strand.

As in most, if not all families of ferns, the gametophyte is capable of regeneration by means of branches which begin from a single cell. The regenerated growths in this family may arise on the margin or on the surface, dorsal or ventral. In the course of time the regenerated branches may bear antheridia while still attached to the parent thallus or after separation. Archegonia are not likely to develop on regenerated branches until after their separation from the parent.

Discussion. In the Gleicheniaceae the gametophyte presents little variation if one may judge from the sampling which has been made in available species of this family now credited with about 130 species (Copeland, 1947). Until something is known of the gametophytes of *Platyzoma* and *Stromatopteris* which are considered to be most closely related to the genus *Gleichenia*, those of the four larger genera will have to stand as the type of the family. Considering the limitations of a slightly differentiated thallus, the gametophyte is almost as distinctive for its generation as is the sporophyte of this well-defined and natural family. The gametophyte can be recognized by external characters: the heavy midrib, the uplifted and more or less ruffled wings; the stiff, brown and abundant rhizoids; the two-celled glandular hairs; the antheridium which is usually large and complicated; and the archegonium with its long or slightly curved neck extending forward. This gametophyte is not to be confused with that of any other group with the possible exception of *Dipteris*—which is very similar except that it has no hairs—and perhaps *Matonia* of which little is known except the prothallus collected by Lang and ascribed to *Matonia* (Bower, 1926).

The position assigned to the Gleicheniaceae as one of the most primitive families of ferns on the basis of sporophyte characters, is also supported by the structures and development of the gametophyte. Copeland (1947) states that the Gleicheniaceae is more advanced than the Osmundaceae and about at the evolutionary level of the Schizaeaceae. The gametophyte while in advance of the Osmundaceae has hardly reached the level of the Schizae-

aceae. In the latter family although the germination habit shows a range in type suggestive of the Gleicheniaceae, the more primitive type —the mass type—is less strongly developed and less common while the filamentous is more strongly developed (Rogers, 1923); the thallus is not so heavy and has a much thinner midrib; and the antheridium is very much simpler in structure of wall and has a much lower sperm output. The thick midrib of the Gleicheniaceae, especially its thickness at the time of the formation of archegonia is in strong contrast with that of higher families. It is common in the higher families for the archegonium initial to be marked out when the thallus is only two cells thick, while four is the usual number in the Gleicheniaceae. Some importance must be attached to the tendency for a wall to form between the neck canal nuclei to make two cells. Although the two-celled canal is by no means as common as the binucleate neck canal, several examples were found in all four genera of this investigation. Campbell, however, said it was the usual type in three of his four species. The presence of a wall has been reported as of occasional occurrence in the Marattiaceae and Osmundaceae, but it has not been reported for higher families. The abundance of chloroplasts in early stages of rhizoids even on old prothalli seems to be an indication of belated differentiation which the author has found to be much more common in primitive families and less frequent or absent in higher families.

The peculiar and characteristic hairs are a specialized feature not found in any other family so far as is known. The presence of the hairs is the chief morphological character distinguishing the Gleicheniaceae from that of *Dipteris*, but it should be added that *Dipteris* has a strong tendency towards apogamy. It would be desirable to know whether the gametophytes of all species of the Gleicheniaceae develop hairs. Although no other primitive family has developed the type of hair which shows the unusual origin from a special initial, the same type of origin is found in a more advanced family, the Cyatheaceae, to which a connecion with the Gleicheniaceae has been attributed by Bower (1926). The large multicellular bristle-like hair of the Cyatheaceae while differing in form and structure, has the same type of origin, arising as it does from a wedge-shaped cell at the anterior face of a cell on dorsal or ventral surface in the archegonial region of the thallus (Stokey, 1930).

It is surprising that apogamy has not been found in this family. In general the ferns of the Gleicheniaceae are found in sunny locations—a type of habitat which is frequently associated with apogamy. The large size and scrambling growth of the leaves may give sufficient protection to the gametophyte to check any manifestation of apogamy. It would seem most likely to occur in the genus *Gleichenia* which embraces most of the smaller and more xerophytic members of the family, but *G. vulcanica* is the only member of

this group in which there has been a study of the large old gametophytes in which apogamy is likely to occur as an occasional phenomenon.

The uniformity of this family as seen in the gametophyte is such that it can make but a limited contribution relative to the line of generic distinction accepted in recent classifications. Differences which may be suggestive are in the thickness of the midrib, the size and complexity of the antheridium, and, perhaps, in the length of neck of the archegonium. There has hardly been sufficient evidence to permit categorical statements, but the indications are that *Gleichenia* does not have as thick a thallus as the other three genera, and that its antheridium has a smaller sperm output and simpler structure of the wall. Campbell found the antheridium of *Dicranopteris pectinata* distinctly smaller than that of *Sticherus laevigatus*, and that of *D. linearis* slightly smaller than that of *D. pectinata*. The genus *Gleichenia* (the old section Eugleichenia) has long been recognized as a specialized xerophytic group. Chrysler (1944) on the basis of leaf anatomy interpreted *Sticherus* as the most primitive genus in the family and *Gleichenia* as the most specialized. The extraordinarily long neck of the archegonium of *Gleichenia* should probably be regarded in the nature of a specialization. The gametophyte, then, not only indicates that the Gleicheniaceae is a natural and primitive family, but that within the family, *Sticherus*, the largest genus, contains the most primitive members and *Gleichenia* the most advanced or specialized, with *Hicriopteris* and *Dicranopteris* at an intermediate stage of advancement; and that *Gleichenia* is farther removed from the other three genera than they are from each other.

SUMMARY

1. The early stage of the gametophyte shows great variation and may be a mass, plate or filament. The first wall divides the germinating spore into the first prothallial cell and the first rhizoid. There may then develop a more or less irregular mass, a plate or a filament of two or three, less frequently eight to ten cells. The young prothallus takes on a spatulate form and then becomes cordate.

2. The mature gametophyte is cordate with wings which have a more or less irregular margin. As the thallus continues growth it becomes ribbon-like with a heavy midrib (10–18 cells thick) and uplifted ruffled wings. The base in large gametophytes is usually heavy and club-shaped. Old gametophytes may branch and maintain two apices producing archegonia. The rhizoids are stiff, abundant, usually reddish brown, and in general limited to the midrib.

3. In many species two-celled glandular hairs develop on the midrib or wings in the region of the archegonia; the large tip cell may contain tannin. The hair arises from a special initial, a wedge-shaped cell cut off from the anterior side of a cell in the region of the archegonia. Occasionally such hairs are also found on the margin.

4. The gametophytes are monoecious. The earliest appearance of antheridia was in *Sticherus bifidus* seven weeks after the planting of spores. The antheridia vary greatly in size from relatively small to large and complex with 10–12 cells in the wall and with a spermatocyte content of several hundred. The cap cell is thrown off at dehiscence. The archegonia have long necks directed towards the apex, ordinarily with seven to nine cells in a tier, but in *Gleichenia vulcanica* sometimes 12–14. The necks are approximately straight or bent forward towards the apex. The axial row usually consists of egg, ventral canal and a binucleate neck canal cell, but the presence of two canal cells in the neck is not unusual.

5. No cases of apogamy were found in any of the ten species in this investigation.

Mount Holyoke College
South Hadley, Mass.

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THE CELL FORMS, AND THEIR COMMON SUBSTANCE REACTIONS, IN THE PARENCHYMA–VASCULAR BOUNDARY[1]

D. S. VAN FLEET

Tissues are identified by their histological location, by the characteristics of their cell walls, and the shape or form of the cells. They may also be identified by the histochemical reactions of the protoplasts and the cell walls. The cells of the boundary tissue between the stele and cortex have many anatomical forms. The typical endodermis may have Casparian strips, U-shaped unilateral walls, suberized walls or a combination of these. In some parts of the plant, however, particularly in the aerial stems and leaves, the cells in the parenchymal-vascular boundary do not have the accepted anatomical characteristics associated with the endodermis. Cells of the parenchymal-vascular boundary, lacking endodermal characteristics, have been generally recognized by plant anatomists as the "border parenchyma," "parenchymatous bundle sheath," "bundle sheath" or "starch sheath," and in the grasses as the "mestome sheath." The purpose of the work presented here is to show that "border" or "sheath" cells give histochemical reactions like those in the endodermis, and that sheathing cells may develop, or be induced to develop, like the typical endodermis.

In preliminary surveys it was found that cells of the border parenchyma gave the same reactions to applied indicators as the endodermis. It was found that all of the cell types in the cortical-vascular boundary of root, stem and leaf react in a characteristically similar way with fat stains, polychrome dyes, oxidation-reduction indicators and other reagents at various stages in their differentiation and maturation. Even in the lower non-vascular plants, in the moss gametophyte stem, the cells between cortex and conducting strands were found to give reactions for the endodermis even though the anatomical signs for this tissue were absent. Based on these preliminary observations an attempt was made to demonstrate endodermal reactions and induce endodermal cell wall formations in cells which are generally classified as "border" or "sheath" cells but not as endodermal cells. It was found that all cells of the parenchymal-vascular boundary have common substance reactions and that they may be induced to develop typical endodermal structures.

Materials and methods. For a study of the border parenchyma surrounding the vascular bundles in leaves and petioles the following plants

[1] Illustration published with the assistance of the Lucien M. Underwood Memorial Fund.

were used: *Raphanus sativus* L. (nine varieties used are listed in the section on results), *Sansevieria zeylanica* Willd., *Senecio obovatus* Muhl., *S. glabellus* Poir., *S. aureus* L., *Lactuca sativa* var. *romana,* Hort. (Cos lettuce), *Brassica rapa* L., *Brassica oleracea,* var. *capitata* L. (Jersey Wakefield and Copenhagen Market), *Helianthus annuus* L., *Prunus Laurocerasus* L., *Billbergia nutans* Wendl., *Smilax hispida* Muhl., *S. herbacea* L., *Allium cepa* L. The boundary tissue cells of the moss gametophyte stem, located between the cortex and the simple conducting strand or stele, were studied in species of *Physcomitrium, Polytrichum,* and *Climacium.* The mestome sheath or bundle sheath of the grasses was examined in *Pennisetum alopecuroides* L., *P. ruppelii* Steud., *P. villosum* R. Br. and *Poa annua* L.; the parenchymal sheath was examined in *Oplismenus hirtellus* L. (Beauv.) and *Zea Mays* L.; the mestome sheath in the sedge *Carex laeviconica* Dewey and in the rush *Scirpus atrovirens* Muhl. was examined.

The reagents, applied to fresh free-hand or freezing microtome sections, were known from previous work to react characteristically and almost exclusively with the endodermis. The indicators used reacted more quickly with the endodermis than with other tissues. Indicators for oxidation-reduction in the endodermis were the following—a solution of gum guaiac (3% in 70% ethanol treated with Norite charcoal to remove peroxides) used with, and without, the addition to the sections of mild alkaline salts (sodium selenite, sodium borate or dibasic phosphate); benzidine (1% in 70% ethanol) and benzidine dihydrochloride (1% aqueous); leuco methylene blue (1% aqueous) reduced by sodium thiosulfate or hydrogen bubbled through platinized asbestos. The use of these indicators for oxidation in the endodermis has been described by Wodziczko (1930), Rajkowski (1934) and Van Fleet (1942, 1943, 1945). Keeble and Armstrong (1912), Sanglet (1928) and Samisch (1935) described methods of demonstrating the oxidation of benzidine and guaiac by the cells around the vascular bundles.

Potassium iodide (1% aqueous and also used as 1% solution in glacial acetic acid) was applied to fresh sections of normal and etiolated material, and it was found that the homologue of the endodermis released free iodine, as indicated by brown stain of plasma and walls, by an oxidation system present in that tissue. The same tissue in the normal plant did not oxidize potassium iodide to free iodine. Although this reagent did not work in all of the plants examined it was included in the survey.

The use of polychrome indicators for demonstrating neutral fats in the undifferentiated and aerial endodermis and for free fatty acids in the differentiated endodermis in subaerial and aerial etiolated stems has been described by Priestley (1926) and Van Fleet (1950). Polychromed Nile blue sulphate contains the blue salt of a red base which forms blue salts with fatty acids, and the red dye particles dissolve in neutral fats coloring them pink or lavender. Fresh sections were treated with 0.1% sodium carbonate and

polychrome Nile blue sulphate in glycerine; the sections were then washed and mounted in glycerine. Methylene blue polychromed with lithium carbonate (.250 gm. dye, 4.0 gm. lithium carbonate in 300 ml. water, heated to 90° C for 10 minutes and acidified with 5 ml. acetic acid) was found to be a more satisfactory reagent than Nile blue sulphate for differentiating neutral fats and free fatty acids. The polychrome methylene blue used was a modification of Goldhorn's polychrome (Van Fleet, 1950).

Several basic dyes (malachite green, neutral red, methylene blue and Nile blue) were used to demonstrate the hydrolysis of fat. The free base of these dyes has a different color from their salts and the dyes were found to differentiate neutral fats from free fatty acids (method of Knaysi, 1941). Malachite green was olive green with neutral fats and blue-green with fatty acids; neutral red was orange-yellow with neutral fats and red with free fatty acids; methylene blue was red or lavender with neutral fats and blue with fatty acids. The dyes used were tested with oleic acid, linoleic acid, soya and corn oils.

The Kreis test for aldehydes in fats and lignin was used to detect the Casparian strip and lignosuberic lamellae in the cells examined. The Schiff reagent for aldehydes was also used. Reduced pararosaniline hydrochloride was used in the Schiff test. The pararosaniline (0.25 gm. in a liter of water) was reduced with sulfur dioxide gas.

Safranin, chrysoidin, gentian violet, osmic acid and the Sudan dyes (Sudan III and IV) were used as general fat stains. The basic stains stain the plasma fat of the endodermis, and osmic acid appears to stain the same constituents. Osmic acid is reduced by oxidizable fats, and it was about the same in reaction intensity as the fat stains. The Sudan stains differentiate the suberized pellicles and lamellae of the endodermis. All of the above were used in a concentration of 1–3% in 70% alcohol with the exception of the osmic acid (1% aqueous).

A dilute aqueous solution of iodine and potassium iodide was used as a starch stain for cells of the starch sheath. It was used where counts were made of the increase or decrease of starch in the border parenchyma or "starch sheath" of etiolated and normal plants.

For a study of control sections the fats were removed from sections by placing sections in pyridine for a short time. Washing sections in alcohol or pyridine interfered with the polychrome dye method of determining whether neutral or free fatty acids were present in a given cell type.

All of the plants listed above were grown under field or greenhouse conditions and then placed under dark cans. Those in the field were covered with dark cans; greenhouse plants in pots were placed in the dark in the laboratory at room temperature and in a cold room at 0°–5° C. Sections of etiolated petioles and leaves were made free-hand or with the freezing microtome at 5 day intervals over a period of 1 to 20 days. Observations

were made of the progressive change of the border parenchyma to the typical endodermis as indicated by anatomical cell-wall changes and changes in histochemical reaction. Normal tissue sections were compared with etiolated tissue sections by using a Bausch and Lomb comparison eyepiece.

Observations were made on anatomical cell wall characteristics and on substance reactions in the cell contents. The cell wall modifications of the typical endodermis (suberin lamellae, Casparian strips, unilateral lamellae, oxidation of indicators by wall contents) were sought in the border parenchyma in aerial parts of etiolated plants. The plasma contents of the same cells were observed for conversion of neutral fats to free fatty acid, change in number of starch granules, oxidation of indicators and general staining of fats with basic stains and osmic acid.

Glucosidase, according to Guignard (1890), is localized in perivascular tissues. He found that glucosides are distributed in all cells but that glucosidase is in the endodermis and pericyclic tissues. In a preliminary survey the writer found the methods of Guignard revealed glucosidase and hydrolyzed aglycone products localized in greatest quantity in the endodermis in seedling stems and border parenchyma of leaves of *Prunus Laurocerasus*. Aglycones, as hydrolized and oxidized tannic and phenolic substances, were found in greatest quantity in the perivascular tissues; in fresh sections the act of sectioning brought glucoside and the hydrolyzing enzyme together. Fresh sections were allowed to stand at room temperature for 10 minutes and 1% ferric chloride was applied to reveal aglycone tannins. Emulsion (glucosidase) in crude form, prepared by the method outlined in Sumner and Somers (1943), was applied to other sections for a similar period and the sections treated with ferric chloride. Stem and leaf sections of both control and enzyme treated sections were compared. Amygdalin (obtained from Bios Laboratories Inc.) was applied to fresh sections, and the Berlin blue and picric acid methods of determining the hydrocyanic acid liberated from the amygdalin were used to determine the distribution of glucosidase. Basic dyes, polychrome dyes, fat and redox indicators were also applied to sections of *Prunus* leaves, stems and petioles to observe similarities and/or differences in border parenchyma and the endodermis.

The localization of aglycone tannins in bromeliads, reported to be primarily in the vascular bundle sheath (Wallin, 1898), was confirmed in *Billbergia nutans* and compared with the tannin content of the endodermis.

Results. The conversion of border parenchyma to endodermis. In a survey of anatomical and histochemical changes in the border parenchyma in petioles of radish plants grown in the dark there was found a change from typical border sheath cells to cells which had the characteristics associated with the endodermis. The border parenchyma cells of the etiolated plants were observed to undergo a series of changes in the plasma and cell walls as summarized in table 1. In general, in two of the varieties used, a

TABLE 1. *Survey of reactions in the border parenchyma of normal, early and late etiolation stages in petioles of Raphanus sativus (var. White Strasburg). The plus signs indicate a positive reaction and an arbitrary estimate of intensity; minus signs show the absence of a reaction, or a very weak reaction that was not obtained consistently.*

		Normal	Etiolated	
		20 days old	5 days	10 days
Guaiac Oxidations	Wall	+	++	−
	Plasma	−	−	+
Polychrome Reactions	Wall			
	Neutral Fats	−	−	−
	Fatty Acids	−	+	++
	Plasma			
	Neutral Fats	++	−	−
	Fatty Acids	−	++	−
Oxidation of Redox Indicators	Wall	−	−	+
	Plasma	+	+	−
Sudan Dyes	Wall	−	+	++
	Plasma	−	+	±
Aldehydes (Kreis & Schiff Reagents)	Wall	−	+	−
	Plasma	−	±	−
Starch Content in Number of Grains		1420	28	0
Casparian Strip		−	+	++

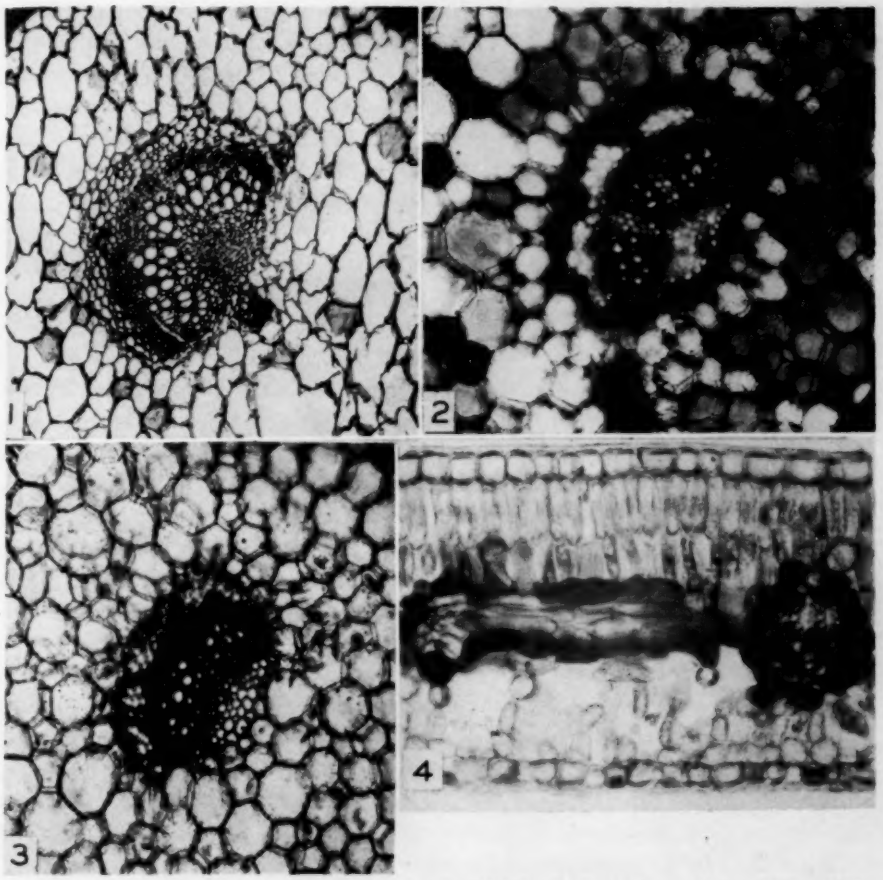
sequence of progressive change in the cells from the normal condition over to the etiolated condition may be summarized as follows: starch hydrolysis, conversion of neutral fats to free fatty acids, change from a condition of reduction to one of oxidation, a gradual deposition of aldehydes and fats in the cell wall and concomitantly a disappearance of fats from the plasma of the cells. The same general trend of reactions and changes in the border parenchyma was observed in fully differentiated tissues as well as differentiating tissues.

The border parenchyma cells in normal petioles stained red with poly-

TABLE 2. *General view of substance and cell-type changes in perivascular tissues accompanying etiolation*

Light		Dark
Cell Types		
Undifferentiated endodermis	→	Casparian type, suberized, or Ø type endodermis.
Simple Casparian endodermis	→	Suberized or Ø type endodermis.
Starch sheath	→	Starch hydrolized → Casparian, suberized, or Ø type endodermis.
Border parenchyma	→	Endodermis with Casparian strips or suberin lamellae.
Substances		
Neutral fat	→	Free fatty acids → Fat oxidation and deposition.
Starch	→	Starch Hydrolysis.
Protoplasmic Fat	→	Cell Wall Fat.
Solvent Soluble Fat	→	Insoluble Fat.
Reduction of Indicators	→	Oxidation of Indicators.

chrome dyes; with etiolation the differentiating and dedifferentiating border parenchyma cells stained blue with the polychrome dyes (figs. 1, 2 and 3). A change from neutral fats to free fatty acids was thus indicated by the



FIGS. 1–4.—FIGS. 1–3. Transections of petioles of *Brassica sativus*, through the median bundle, showing Nile blue sulphate reactions for neutral fats and free fatty acids, ×300. FIG. 1. Section of normal illuminated petiole cut on freezing microtome and stained with light green and safranin. FIG. 2. Nile blue sulphate reaction in free-hand section of normal illuminated petiole. The border parenchyma cells are heavily stained with red dye particles; some of the cortical cells also contained neutral fats as indicated by red dye particle absorption. FIG. 3. Nile blue sulphate reaction in free-hand section of etiolated petiole. The border parenchyma cells stained blue indicating free fatty acids; the weak staining reaction is characteristic of fully etiolated tissue. Neutral fats are not present and free fatty acids are found, as indicated, only on the phloem side of the bundle. Neutral fats and free fatty acids are not detectible in parenchymal cells. Blue dye particles strongly absorbed by xylem in both normal and etiolated petioles. FIG. 4. Cross-section of portion of leaf of *Prunus Laurocerasus* showing absorption of the basic dye safranin by the bundle sheath. The same cells contained glucosidase as demonstrated by amygdalin hydrolysis using Berlin blue and picric acid methods of detecting liberated hydrocyanic acid, ×300.

polychrome dyes. Control sections of both normal and etiolated petioles, treated with pyridine for 24 hours to remove fats, did not react with polychrome dyes.

It was found that in the normal border parenchyma cell the presence of neutral fats and the failure to oxidize indicators occurred simultaneously, and as free fatty acids began to appear in border parenchyma cells in etiolated petioles the redox indicators were readily oxidized. The guaiac plasma reaction was weak in the normal plants and strong in the etiolated, being strongest in the early stages of etiolation and zero toward the end. The oxidation of guaiac to guaiac blue became stronger in the walls of the cells with increase in duration of the period of etiolation. There was an increase in deposition of suberin in the walls of the cells in the etiolated petioles as shown by Sudan dyes and iodine staining. The Kreis and Schiff tests for aldehydes were positive in either the wall, or plasma, or both, with the increase in duration of etiolation. The starch content dropped to zero in 10 days of etiolation; by the time well-defined suberin lamellae could be detected the starch content was zero. The White Strasburg and the White Icicle varieties developed Casparian strips in the parenchymatous sheath cells opposite the phloem in 10 days of etiolation, but the Casparian strip did not develop in the other 6 varieties used (Long Scarlet, Early Scarlet Globe, French Breakfast, Crimson Giant, Red Early Scarlet and Chinese Rose). Several varieties were used merely to establish the fact that there is variation within a species in the development of this tissue. In general the fat content and oxidation of indicators appeared to be higher in the two varieties that developed Casparian strips, but no quantitative measurements could be obtained with a photometer. Variations in thickness of sections and variations in petiole anatomy made it difficult to get reproducible photometer readings in projected sections of the various petioles.

The fully differentiated border parenchyma cell in the normal leaf dedifferentiated to the typical endodermal cell type with increasing etiolation in the leaf. The fully matured border parenchyma cells rarely developed Casparian strips when plants were held in the dark, but there was starch hydrolysis, gradual change from neutral fats to free fatty acids and deposition of fats in cell walls. The border parenchyma cells of young leaf-petioles that differentiated under conditions of etiolation developed like typical endodermal cells. The Casparian strip was well defined in the border parenchyma cells that differentiated in leaves that developed in the dark.

There was an induction period in the conversion of neutral fats to free fatty acids as the normal border parenchyma changed to the endodermal cell type. The induction period was short in the White Icicle variety (48 hours) and much longer (3–5 days) in other varieties. When neutral fats were being converted to free fatty acids, oxidation of redox and guaiac indicators was very weak; but after free fatty acid reactions were strongly

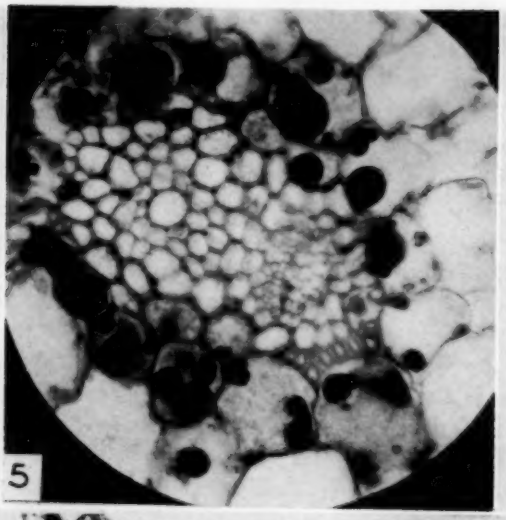
defined the oxidation of indicators was also clearly evident.

In other plants examined the same general results were obtained. *Senecio obovatus, S. glabellus* and *S. aureus* were taken from the field and placed in dark cans, and the differentiation of the border parenchyma was observed at intervals in etiolated petioles and leaf blades. In *Senecio glabellus* the development of well defined Casparian strips was observed, but in *S. obovatus* the Casparian strips did not develop and in *S. aureus* the strips were sporadic and difficult to detect. In *Helianthus annuus, Sansevieria zeylanica, Smilax* (*S. herbacea* and *S. hispida*) and *Allium cepa* the polychrome reactions were less sharply defined, but other histochemical and anatomical results were as in radish. In *Lactuca sativa, Brassica rapa, Brassica oleracea* var. *capitata* the reactions in the normal tissue indicated neutral fats and the reactions in the etiolated were sharply defined for free fatty acids and oxidation. *Lactuca* and *Brassica* were more nearly like the radish in histochemical reaction than any of the other species examined and gave very clearly defined reactions in both etiolated and normal tissue.

Glucosidase localization in the parenchymal-vascular boundary. In *Prunus Laurocerasus* it was found that there is a general distribution of the glucoside prunasin in all parenchymal and dermal cells. Glucosidase activity, as revealed by tannin hydrolysis and oxidation, was localized primarily in the endodermis of the stem and root and border parenchyma of the leaves. A similarity in free tannin content in the endodermis and border parenchyma was observed.

Amygdalin was hydrolized strongest in the border parenchyma as indicated by the picric acid and Berlin blue methods of detecting hydrocyanic acid. It was found that basis dyes (chrysoidin, aniline green, methylene blue and safranin) were strongly absorbed by the perivascular tissues in leaf, petiole and stem. Polychrome dyes and reduced redox indicators reacted in a similar way in the parenchymal-vascular boundary in young leaves and stems in border parenchyma and endodermis. The strong absorption of basic dye and high tannin content in the border parenchyma is demonstrated in figure 4. The localization of oxidized aglycone tannins in the parenchymal-vascular boundary in leaves and stems of *Billbergia nutans* was confirmed (Wallin, 1898). The endodermis and the border parenchyma or "vascular-bundle sheath" as Wallin called it, reacted similarly with ferric chloride to show a very high tannin content as compared with other cells (fig. 5).

The parenchymal-vascular sheath in the moss gametophyte stem. Lorch (1931) and von Guttenberg (1943) describe the "endodermis" of the gametophyte stem as a *Schutzscheide, Starkescheide* or *Endodermis*. In the reviews by Lorch and von Guttenberg there are references to characteristics one associates with the typical endodermis; radial wall deposition as in the typical Casparian strip (Coesfeld, 1892), localization of starch, affinity for fat dyes, resistance of the cell walls to acid hydrolysis.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The application of polychrome dyes to fresh sections of the gametophyte stem of *Polytrichum, Physcomitrium* and *Climacium* revealed neutral fats in the perivascular sheathing cells in young tissue from above the ground line; in older portions of the stem, and in the below ground stem and rhizome, reactions were obtained for free fatty acids. The reaction for neutral fats was transitory. In methylene blue polychromed with lithium carbonate, transitory and very small neutral fat globules were observed to hydrolize in the reagent to give a reaction for free fatty acids. The plasma of these cells strongly absorbed basic stains (fig. 6). When sections were held in pyridine or acetone for a few days the removal of fat was sufficient to interfere with fat staining. Very mild oxidation of guaiac was induced by pretreatment of sections with 1% sodium selenite, and the mild oxidation of reduced indicators was observed in some moss stems. The oxidation of indicators was very weak in the endodermis, but it was stronger in this tissue than in the cortex or stele.

Endodermal characteristics observed in the mestome sheath. The mestome sheath in the grasses was originally described by Schwendener (1890). He inferred that it was a bundle sheath similar to the endodermis. An attempt was made in the work reported here to show that the mestome sheath is a type of endodermal sheath. It was found that some or all of the attributes of a true endodermis may be detected in the mestome sheath. In the young differentiating leaf of *Scirpus atrovirens* it was found that the mestome sheath cells developed Casparian strips. In older leaves the Casparian strip stage in differentiation was masked by unilateral deposition that gave the typical U-shaped appearance to the cells. Hydrolysis of the free-hand sections with dilute hydrochloric acid revealed the bands of resistant Casparian deposits in the radial walls of young cells. The mestome sheath of *Pennisetum villosum, Carex laeviconica* and *Oplismenus hirtellus* also developed Casparian deposits in the radial walls prior to the unilateral deposition characteristic of the mature mestome sheath. Hydrolysis of sections with dilute sulfuric acid revealed Casparian-like deposits in other *Pennisetum* species (*P. ruppelii, P. alopecuroides*). In *Oplismenus hirtellus* the entire radial wall was modified to give the appearance of a broad but poorly defined Casparian strip.

The rapid oxidation of guaiac and benzidine by the developing mestome sheath was observed in *Poa annua, Pennisetum* spp., *Carex laeviconica* and *Scirpus atrovirens*. In *Zea Mays*, which lacks a mestome sheath, the cells of the parenchymal-vascular boundary did not oxidize these indicators. Re-

FIGS. 5–6. FIG. 5. Transection of portion of mature leaf of *Bilbergia natans* through a single major bundle showing localization of oxidized tannins in the border parenchyma. ×300. FIG. 6. Transection (made by Dr. A. M. MacEwan) of gametophyte stem of *Physcomitrium* showing characteristic absorption of basic dyes (safranin) by the cells of parenchymal-vascular boundary. ×300.

duced methylene blue and reduced 2–6 dichlorophenol indophenol were readily oxidized by differentiating mestome sheath cells, but the layer of cells in the same histogenic position in corn leaves did not oxidize these redox indicators.

The mestome sheath had, at some stage in its development, the characteristics of the endodermis. These characteristics were not found simultaneously in any one sheath, but they appeared at various stages in development in at least some of the cells of the sheath. The Casparian strip was found in some of the mestome sheath cells. The localized and immediate oxidation of redox indicators was observed in mestome sheath cells. The reaction with polychrome methylene blue in young mestome sheath cells was similar to reactions observed in the young cells of the endodermis. The U-shaped cells characteristic of the endodermis were strongly developed opposite the phlom. The endodermal characteristics found in the mestome sheath were always more pronounced opposite the phloem than opposite xylem.

In addition to the mestome sheath cells there were parenchymal sheath cells surrounding the bundle in leaves of the grasses and sedges. The parenchymal sheath was outside, and surrounding, the mestome sheath. The parenchymal sheath cells contained some chloroplasts and did not exhibit the characteristics of the endodermis. In those grasses lacking a mestome sheath there was present a single layer of parenchymal sheath cells. The parenchymal sheath in corn leaves, lacking a mestome sheath, did not have the typical endodermal characteristics unless the plants were etiolated.

In a variety of *Oplismenus hirtellus* with variegated leaves, the sheathing cells in the variegated areas lacking chlorophyll exhibited mestome sheath and endodermal characteristics. In the green areas of the leaves the the sheathing cells were of the parenchyma sheath type and lacked mestome sheath and endodermal characteristics. Albino corn seedling leaves were examined and compared with leaves of comparable age in normal corn plants, and it was found that the cells of the bundle sheath in the albino plants gave more marked reactions with redox and fat indicators than in normal plants. Albino and non-green areas in the leaves examined had sheathing cells like those observed in etiolated plants. In anatomical appearance and in histochemical reactions the sheathing cells were similar to bundle sheath cells in etiolated leaves.

The dedifferentiation at season-end of border parenchyma to the typical endodermis. Parenchyma sheath cells suberize at season-end, in leaves and aerial stems in the late stages of maturation, to become suberized bundle sheath cells. Scott, et al (1948) described suberization of the bundle sheath in maturing orange leaves and observed that "the major veins are inclosed in a suberized envelope". Warden (1935) observed the season-end differentiation of the endodermis in stems and flower peduncles of *Senecio vulgaris*. Observations were made on *S. obovatus*, *S. aureus*, and *Zinnia* confirming

the observations of Warden. Season-end differentiation and suberization of the bundle sheath was observed in herbaceous *Smilax* (Van Fleet, 1949).

Discussion. It is apparent that the cells of the border parenchyma or bundle sheath give either histochemical reactions like those characteristic of the typical endodermis, or they may be induced, by etiolation, to give reactions like those observed in the endodermis. Border parenchyma tissues lacking the anatomical characteristics of the endodermis readily develop Casparian strips and fatty deposits in the cell walls when the leaves or stems are held in the dark. When below-ground or subaerial environmental factors prevail one finds typical endodermal characters being expressed. The cells of the parenchymal-vascular boundary may differentiate as "border parenchyma", "bundle sheath" or "starch sheath" cells, but they are always potentially endodermal cells. All of various types of sheathing cells have common stages in differentiation in which the marked histochemical and anatomical features of the endodermis may be revealed or detected. Summarizing his work on the Vicieae Bond (1935) has aptly stated, in agreement with Priestly and Ewing (1923), "the shoot of these plants is potentially an endodermis-forming organ, but only in darkness is this potentiality realized". The potentiality for endodermal development apparently includes leaves as well as stems as shown in the work presented here.

Whether the anatomical characteristics for the endodermis are revealed or not it must be conceded that the cells of the parenchymal-vascular boundary are potentially endodermal cells even though anatomically it may be convenient to classify them as "border parenchyma", "bundle sheath", etc. The cells occupying this unique and differential position may be identified as endodermal cells by means of their substance reactions. All of the various cell types in the cortical-vascular interface of root, stem or leaf react similarly, at comparable stages in differentiation, with various fat stains, polychrome dyes and oxidation-reduction indicators.

SUMMARY

The cells of the boundary tissue between cortex and stele, between the bundle and surrounding parenchyma, have several anatomical forms. The typical endodermis may have Casparian strips, U-shaped unilateral walls, suberized walls or a combination of these. In some parts of the plant, however, particularly in the aerial stems and leaves, the cells in the parenchymal-vascular boundary do not have the accepted anatomical characteristics associated with the endodermis. These cells of the vascular or bundle sheath, lacking endodermal characteristics, have been generally recognized as the "border parenchyma", and as the "mestome sheath". It was found that all of these cell types contain substances which give histochemical reactions like those obtained in the endodermis, and that these cells may develop, or be induced to develop, like the typical endodermis. All of the cell types in the

parenchymal-vascular boundary of root, stem or leaf react in a characteristically similar way with fat stains, polychrome and basic dyes, aldehyde indicators, and oxidation-reduction indicators. The border parenchyma cell in leaves was found to differentiate and/or dedifferentiate as a typical endodermis under conditions of etiolation. A sequence of change was observed in border parenchyma cells, from the normal to the etiolated condition, as follows: starch hydrolysis, conversion of neutral fats in the plasma to free fatty acids, a change from a condition of reduction to one of oxidation, a gradual deposition of aldehydes and fats in the cell wall and concomitantly the disappearance of fats from the plasma. Glucosidase was found to be localized in the typical endodermis and also in the undifferentiated border parenchyma of the same plants. The parenchymal-vascular sheath in the moss gametophyte stem gave the reactions for an endodermis.

DEPARTMENT OF BOTANY, UNIVERSITY OF MISSOURI,
COLUMBIA, MISSOURI

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GROWTH AND DEVELOPMENT OF THE SHOOT OF GNETUM GNEMON L. I. THE SHOOT APEX AND PITH[1]

MARION A. JOHNSON

The shoot apex of gymnosperms has been intensively studied since Hofmeister (1857) first assumed that the primary tissues of both vascular cryptogams and phanerogams were derived from an apical cell. This assumption was soon refuted by many investigators and Koch (1891) was able to demonstrate conclusively that the shoot apex of the conifers consisted of (a) an outer zone from which epidermis, cortex, procambium and leaf primordia were derived and of (b) an inner zone which produced the pith. A second and quite different type of zonation was discovered by Foster (1938) in *Ginkgo*. Here the major portion of the apex was readily traceable to a unique group of central mother cells. Comprehensive summaries of the literature up to 1939 are to be found in the papers of Koch (1891), Schüepp (1926), Korody (1937), and Foster (1938). Subsequently Foster (1939, 1940, 1941, 1943), Cross (1939, 1941, 1942, 1943a, 1934b), Kemp (1943), Johnson (1939, 1944), Sterling (1945, 1946) and Gifford (1943) have shown that the gymnospermous apex possesses variations in architecture ranging from the massive, zoned apices of the cycads to the smaller apices of the higher gymnosperms in which the trend is toward the elimination of periclinal divisions in surface layer and flanking layers. Cross (1943b), in comparing the apices of the Taxodiaceae with those of the angiosperms states: "The shoot apices of the Taxodiaceae differ from those of the Angiosperms with respect to only one essential feature, viz., the frequency (greater) with which periclinal divisions occur in the surface layer of the summit." The closest approach to complete elimination of periclinal divisions in the superficial layer has been reported from the Gnetales by Gifford (1943) for *Ephedra altissima* Desf.; here one such division was found in a critical study of seventy stem tips.

Little has appeared in the literature on the shoot apex of *Gnetum*. Bower (1882), in a paper largely devoted to the germination and embryogeny of *Gnetum gnemon*, observed that while the apex of dormant buds was clothed with a discrete superficial layer that of seedlings was frequently occupied by a single large cell and that the superficial cells on the flanks of the apical cone frequently divided periclinally. Haining (1920), however, in a study on the development of the embryo found that the cotyledons, hypocotyl, and

[1] Illustrations published with the assistance of the Lucien M. Underwood Memorial Fund.

stem apex possessed a discrete superficial layer. Pearson (1929), in his classical work on the Gnetales, dismisses the shoot apex with the statement: "In *Gnetum* the stem apex is massive," while earlier Duthie (1912) had concluded that: ". . . in every case a small-celled meristem was found" at the tip of the stem of *Gnetum africanum*.

Materials and methods. Eighty five terminal buds were prepared for study. These were collected in active stages of growth as well as in the dormant condition from a potted plant at the Brooklyn Botanic Garden and from an established plant, twenty feet tall, growing free in the soil of the palm house at the New York Botanical Garden.

Formalin-acetic-alcohol (5 cc. formalin, 5 cc. glacial acetic acid, 90 cc. 50% alcohol) and formalin-propronic-alcohol (5 cc. propronic acid substituted for glacial acetic acid in the previous mixture) were employed as killing and fixing agents. All material was embedded in Tissuemat (m.p. 56–58° C.) following dehydration and clearing with ethyl alcohol and xylol respectively or dehydration with tertiary-butyl alcohol. Sections were cut from 5 to 8 microns in thickness and stained in Heidenhain's iron-alum haematoxylon and safranin.

General features of the shoot. *Gnetum gnemon* is a tree from tropical Asia possessing strong upright branches and a profusion of pendant shoots upon which most of the foliage is borne. The leaves are decussate although they are frequently brought into the two-ranked condition by distortion in the internodes.

Dormant terminal buds are effectively enclosed by the snug fitting petiolar bases of the final pair of mature leaves (fig. 11). Each bud consists of: (a) a shoot apex which is free of bud scales but is closely invested with from one to three pairs of leaf primordia or a single pair of leaves about two millimeters in length, (b) two to four axillary apices, and (c) numerous multicellular hairs which grow upward from the leaf axils.

The shoot apex is a blunt cone (figs. 1, 11) with an elliptical base and a tip which may be either circular or elliptical (fig. 2) in transverse section. The apex, as in other opposite-leaved plants (Schmidt, 1924; Louis, 1935), undergoes a rhythmical series of change in size from minimum to maximum to minimum which is correlated with the initiation and growth of the foliar buttresses and their associated leaf primordia. When the apex has attained maximum size, a swelling develops at each end of the major axis of the ellipse involving the lower half of the apical cone (figs. 4, 5, *fb*). Growth spreads laterally from each center of initiation until a collar-like foliar buttress is formed. The initiating loci maintain their early lead in growth and develop as a pair of leaf primordia (figs. 6, 7, *lp*). With the initiation of the foliar buttresses the apex passes from maximum size to minimum size and gradually builds up to maximum size again, but with the new major axis rotated through 90 degrees from that at which the former pair of leaf

primordia originated. There is little increase in the length of the new minor axis following the change in the apex from maximum to minimum size. The new major axis, however, more than doubles its dimensions before the apex attains maximum size during the next plastochron.

Measurements of the apex were made in terms of height of the apex above the first pair of foliar buttresses, and the respective diameters of the major and minor axes of the elliptical base. The method employed was to use serial longitudinal sections cut tangent to the edge of the first visible pair of leaves below the apex of actively growing shoots or to the first pair of adult leaves of dormant shoots. The median section from such a series will show the first leaf or leaf primordium, if it is taller than the apical cone, detached or nearly detached from the shoot as seen in figure 11. This not only frees the apex for direct measurement but since *Gnetum* has decussate phyllotaxis, the next pair of foliar buttresses if present, will be visible in profile view (figs. 4, 5). If the foliar buttresses have not appeared, measurement of the major axis is possible. The length of the minor axis, when the apex was at maximum size, was obtained by counting the number of sections between the axils of the first pair of leaves or leaf primordia. The following dimensions are averages based upon measurements from ten apices which were considered to be at maximum size immediately before the formation of the foliar buttresses: major axis 211 μ, minor axis 155 μ and height 83 μ. The smallest apex observed, also at maximum size, had a major axis of 155 μ, a minor axis of 112 μ and a height of 78 μ. When the apex passes from maximum to minimum size, during the production of the foliar buttresses, its dimensions are reduced to approximately one-half their former size.

The general architecture of the apex can be described best in terms of the tunica-corpus concept as proposed by Schmidt (1924). The corpus, however, possesses a zonal structure which seems best described in terms of sub-apical initials, central mother cell zone, flanking layers and pith rib meristem.

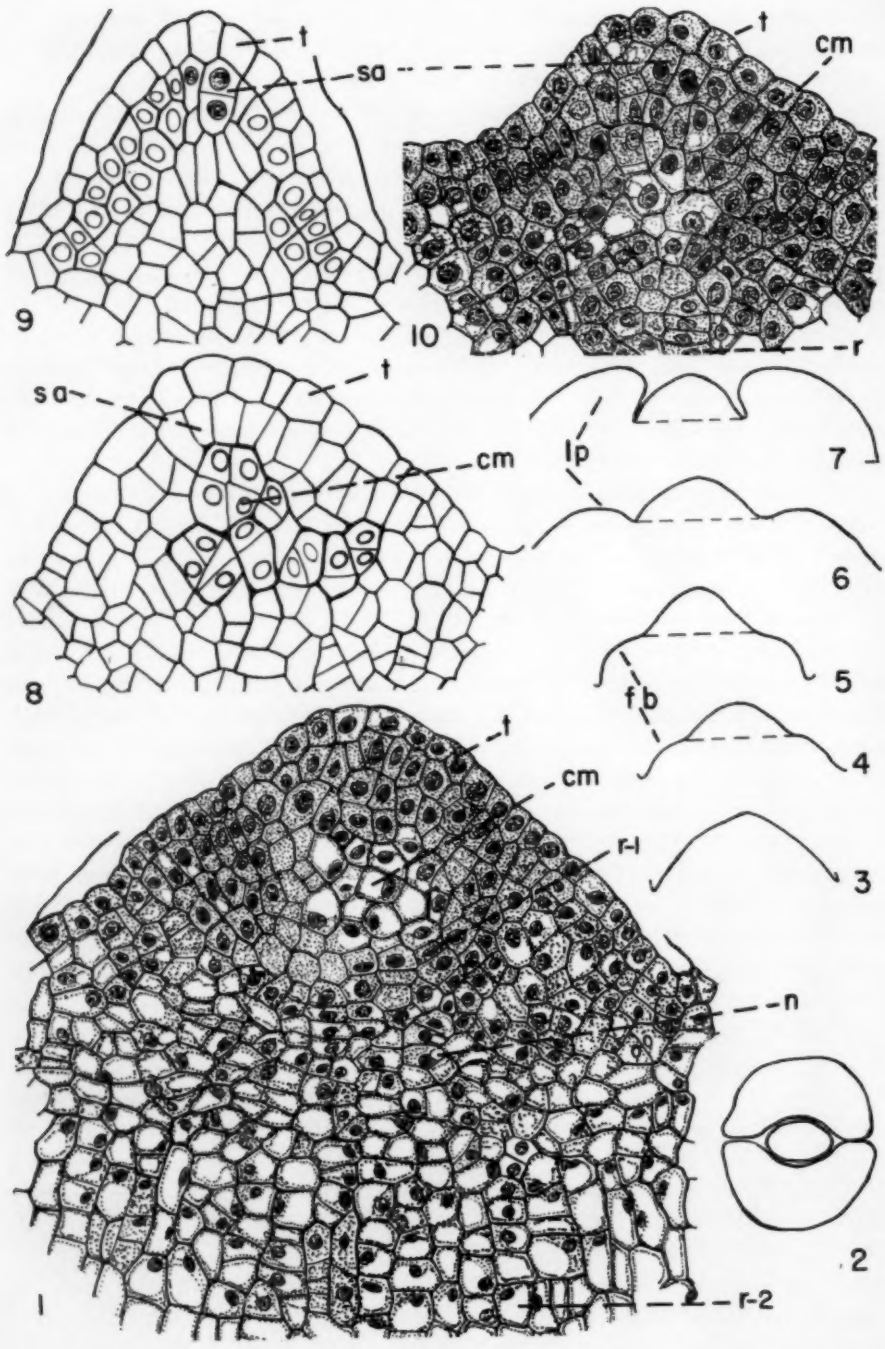
Tunica. The tunica consists of a single layer of cells extending from the axils of the first pair of leaf primordia over the shoot apex (figs. 1, 8, 9, 10, *t*, 12). The cells at the summit are generally somewhat larger, in both diameter and depth, and are often more vacuolate than those on the flanks of the apex which suggests that they may divide less frequently than the flanking cells. Eighty five apices were examined without discovering a periclinal division or evidence of periclinal divisions in the tunica clothing the summit of the dome regardless of whether the shoots were dormant or in an active stage of growth. Periclinal divisions do occur in the tunica during the initiation of the foliar buttresses and rarely (observed in one apex) somewhat higher on the flanks of the apical cone which could not be associated with the formation of the foliar buttresses.

Corpus. Sub-apical initials. A group of sub-apical initial cells occupies the summit of the corpus. These are generally two or three layers in depth and have their long axis parallel to that of the shoot (figs. 8, 9, 10, *sa;* 12). The entire corpus can be traced to divisions in this region. Periclinal and oblique divisions contribute cells to an underlying central mother cell zone while anticlinal divisions add cells to the flanking layers of the corpus.

Flanking layers. The flanks of the corpus are composed of two or more layers of active cells which have been derived from two distinct sources. The principal source is from anticlinal divisions in the sub-apical initials as previously mentioned, however, anticlinal divisions in the periphery of the central mother cell zone make considerable contribution especially in large apices. Division in the flanking layers may be in any plane, but judging from the high degree of stratification, anticlinal seems to predominate (figs. 1, 8, 9, 10). Division in the flanks adds to the leaf buttresses and ultimately contributes to the leaf primordia.

Central mother cells. A zone of central mother cells underlies and is derived from the sub-apical initials. It may lie entirely within the apical cone (figs. 1, *cm;* 12) or within the first pair of foliar buttresses when the apex is near minimum volume (fig. 10, *cm*). Division may occur in any plane with the resultant daughter cells assuming an irregular pattern (figs. 1, 10). This, however, is not a constant feature and may be replaced by a close approach to vertical alignment indicating a preponderance of periclinal over anticlinal divisions. In some instances the central mother cell zone seems to be composed of blocks of cells, i.e., several daughter cells appear to be enclosed within the wall of an original mother cell (fig. 8). Where three such mothercells make contact, the walls may be somewhat thickened. In the majority of the apices examined many of the mother cells were conspicuously vacuolate (figs. 1, 10, 12), while in others this feature was lacking. The absence of large vacuoles could not be correlated with any visibly recognizable condition. It was noted, however, that when vacuolation was well developed in the central mother cell zone of the terminal apex, the same condition was found in the lateral apices of that bud.

Pith rib meristem. Apices at minimum volume, i.e., immediately following the elevation of a pair of leaf primordia, may not possess rib meristem above the first node, in fact, the corpus at this time is reduced to a relatively shallow cap of sub-apical initials and a few central mother cells. Figure 10 shows an apex at nearly this stage; a single file of rib meristem is evident at *r*. As the apex increases in size, the vertical derivatives from the sub-apical initials become incorporated into the central mother cell zone while the cells in the lower portion of this zone give rise to a shallow zone of rib meristem generally not more than two files of cells in depth with each file usually not more than five cells in height (fig. 1, *r-1*). During subsequent growth of the leaf primordia, the vertical stratification of this shallow rib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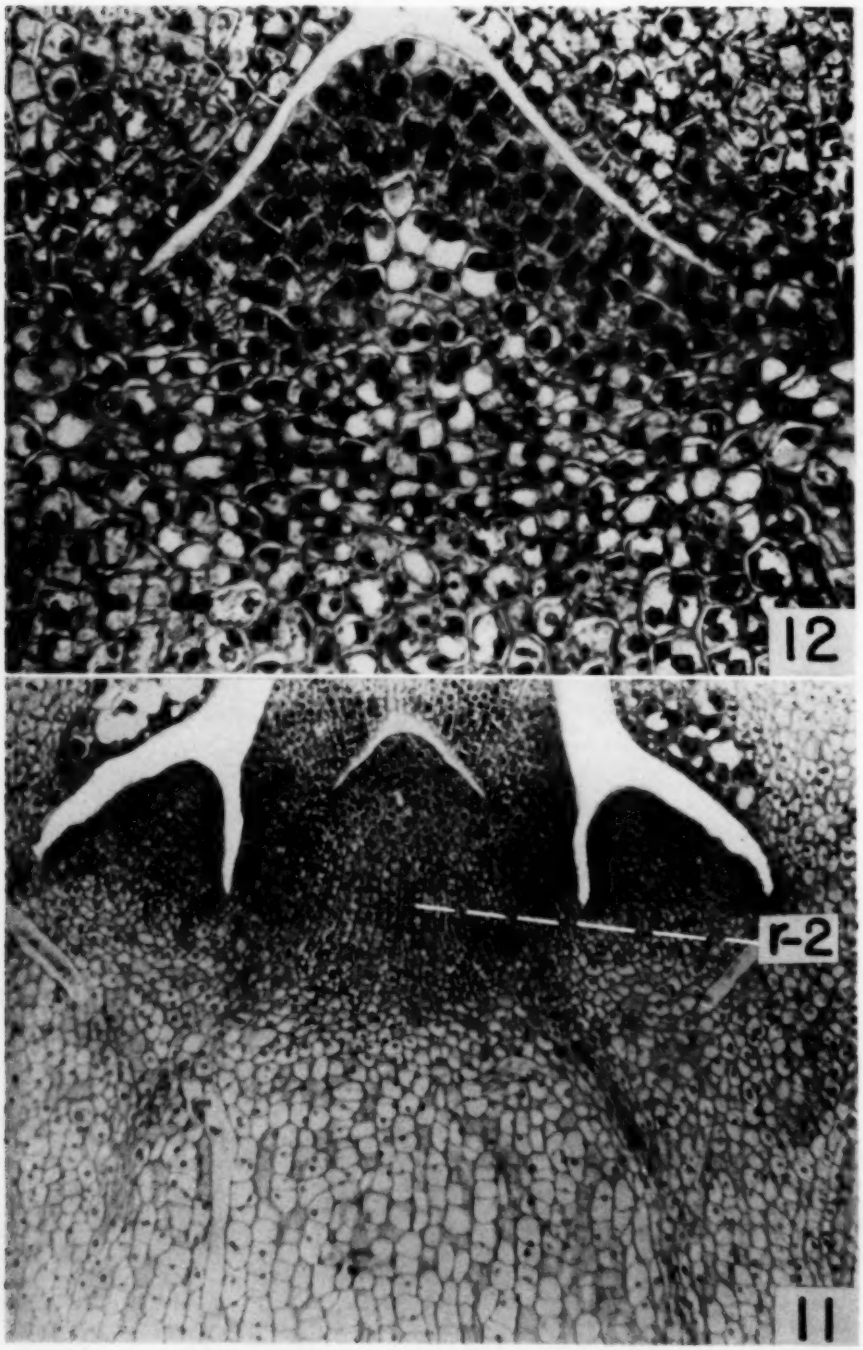
meristem is interrupted by radial enlargement and divisions anticlinal to the summit of the apex. Later, periclinal divisions become more frequent than in any other plane and a rib meristem is reconstituted in what is now the first internode as may be seen in figures 1 and 11, *r-2*. Many of the cells derived from anticlinal divisions during the disruption of the original rib meristem serve as initials for a greatly increased number of new files in the reconstituted rib meristem. The cells in *r-2* are much longer and more highly vacuolate than the low, flat, densely protoplasmic cells of *r-1* (fig. 1). Occasional anticlinal divisions may augment the rib meristem at any time in its development through the production of initials which establish new vertical files (fig. 1, *r-2*).

The relative magnitude of periclinal cell division in the pith rib meristem of the first internode may be estimated readily by determining the length of such an internode in terms of number of rib meristem cells and that of a mature internode in terms of number of pith cells. A first internode somewhat smaller than that shown in figure 11 was selected which was nine rib meristem cells long (total length 122 μ) and a mature internode some 3060 pith cells long (total length 177 mm.). Each cell of the rib meristem would therefore, on the average, produce a total progeny of 340 cells.

The second internode, depending upon its age, may show either (1) even more highly vacuolate but still actively dividing cells than in the first internode or (2) maturing pith with prominent pit fields. Dividing nuclei are generally suspended in shallow bridges of cytoplasm, the phragmasome, as described by Sinnott and Bloch (1941) for mitosis in vacuolate cells. The central region of the stele which will ultimately mature as pith is from two to three times the diameter of that in the first internode. This has been accomplished by an increase in both the number of cell files and in the diameter of the individual cells. An abundance of small intercellular spaces are now apparent as well as scattered, conspicuously branched sclerids.

Discussion. The position of the Gnetales in the plant kingdom, whether they be regarded as representing a Gymnosperm-Angiosperm alliance (Thompson, 1916; Pearson, 1929) or as a highly evolved group which, independent of angiosperm affiliation, has attained many angiospermous characteristics, demands that the group receive critical study and comparison

FIGS. 1–10. FIG. 1. Longisection through major axis of apex at maximum size; ×370. FIG. 2. Transection through apex; ×48. FIGS. 3–7. Longisections cut tangent to the edge of the second pair of leaves. FIG. 3. Major axis of apex at maximum size; ×111. FIG. 4, 5. Minor axis of apex at broken line with foliar buttresses, *fb*, below; ×111. FIG. 6, 7. Elevation of leaf primordia, minor axis at broken line; ×111. FIG. 8. Longisection through major axis of apex near maximum size; ×370. FIG. 9. Longisection through minor axis of apex; ×370. FIG. 10. Longisection through minor axis of apex near minimum size; ×370. Legends for drawings: *cm*, central mother cells; *fb*, foliar buttress; *lp*, leaf primordium; *n*, node; *r-1*, rib meristem which takes its origin from the periphery of the central mother cell zone; *r-2*, reconstituted pith rib meristem in the first internode; *sa*, sub-apical initials; *t*, tunica.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r-2
11

with the angiosperms and other gymnosperms. The value to be placed upon the shoot apex in possible phylogenetic arrangements is still an open question, yet comparison of the role and structure of the shoot apex at various levels in the plant kingdom may be expected to reveal trends which might otherwise be overlooked.

Although Pearson (1929) considered the apex of *Gnetum* to be massive, a comparison with the data on the apices of seed plants as compiled by Kemp (1943) and Foster (1949) indicates that the apex of *Gnetum* at maximum size has a greater diameter (major axis) than in investigated angiosperms (90 μ to 200 μ) and that at no time does it approach that of certain palms and cacti (500 and 700–900 μ respectively) nor those of the cycads which attain a diameter of 3.5 mm. in *Cycas revoluta*. The ratio of height to diameter in *Gnetum* is roughly 1 : 3 and compares favorably with that in the Coniferales and *Ephedra altissima*.

Gnetum, when treated as a greenhouse subject, does not appear to undergo seasonal changes in shoot development as have been reported for an increasing number of species among both gymnosperms and angiosperms. The definite change from maximum to minimum size is correlated with the formation of the foliar buttresses and the subsequent growth of the apex. These rhythmical changes are similar to those described by Schmidt (1924) for opposite-leaved plants under the terms of "maximal area" and "minimal area." Dormant buds in *Gnetum* may contain apices in any stage of development from minimum to maximum size. Zonation is considerably reduced at minimum size, a condition found to exist in *Torreya* by Kemp (1943) and in *Abies concolor* by Korody (1937).

The observations reported in this paper not only confirm Bower's (1882) discovery that the apex of dormant shoots of *Gnetum gnemon* possess a discrete superficial layer but extend it to actively growing shoots as well. It should be pointed out, however, that while periclinal divisions do not occur in the superficial layer at the summit of the apex, they rarely are found in the surface layer on the flanks of the apical cone. This condition is the reverse of that described for the Taxodiaceae (Cross 1943b) and for *Torreya* (Kemp, 1943) where the tendency is to eliminate periclinal divisions in the superficial layer on the flanks but to retain them at the summit except in permanent shoots of *Taxodium distichum* (Cross 1939) and *Cryptomeria japonica* (Cross, 1941). Also in this connection Bower (1882) has reported that the superficial layer is not discrete in the seedling apex although Haining (1920), on the contrary, has found that it is discrete in the embryo of

FIGS 11–12. FIG. 11. Longisection cut parallel to the surface of the first pair of young leaves. The shoot apex is at maximum size and is cut through the major axis. Note the developing axillary buds and reconstituted pith rib meristem, *r-c*, ×108. FIG. 12. Longisection through minor axis of apex. Note the conspicuous vacuolation in several of the larger central mother cells, ×422.

G. gnemon. Furthermore it has long been known that periclinal divisions occur in the surface layer of the nucellus of *G. gnemon* (Strasburger, 1879) and likewise in that of *G. verrucosum* and *G. funiculare* (Karsten, 1893). These variations, particularly those found in the shoot apex of the adult, are no greater than those occurring in angiospermous apices (Boke, 1940, 1947; Reeve, 1948; Engard, 1944; Sharman, 1940, 1943; and Sterling, 1944) which at present (Foster, 1949) are generally described in terms of the tunica-corpus concept of Schmidt (1924). It would seem, therefore, that the tunica-corpus concept can be properly applied to the shoot apex of *G. gnemon*.

Data are not available for the relative frequency of plane of division in the apices of seedlings as compared to adult plants so it is impossible to estimate the weight which should be placed upon Bower's report of periclinal divisions in the surface layer of the seedling apex; it could conceivably represent the recapitulation of an ancestral condition.

The presence of a discrete superficial layer clothing the summit of the apex in *G. gnemon*, while of rare occurrence among gymnosperms, is shared by *Ephedra altissima* (Strasburger, 1872; Gifford, 1943), *Araucaria brasiliana* (Strasburger, 1872), by the permanent shoots of *Taxodium distichum* (Cross, 1939) and to a lesser extent by *Cryptomeria japonica* (Cross, 1941). The four families represented by these genera, viz. Gnetaceae, Ephedraceae, Araucariaceae and Taxodiaceae, undoubtedly have long been distinct so that their possession of a discrete surface layer at the summit of the apex is probably best regarded as having resulted from parallel evolution. The presence of periclinal divisions in the surface layer of the apex is a major trend in the growth of both pteridophytes and gymnosperms. The elimination of such divisions represents a minor trend in gymnosperms but assumes the importance of a major trend in angiosperms and especially in the dicotyledons. The shoot apex of *Gnetum*, therefore, has attained the angiospermous level with respect to the discreteness of the surface layer.

Little is known about the shoot apex in *Welwitschia*, the remaining genus of the Gnetales. Bower (1881) states "In longitudinal sections the epidermis does not appear as a regular layer covering the apical cone." This would seem to imply that the superficial layer is not discrete and can not, therefore, be considered to have attained the status of a tunica as is the case for both *Ephedra* and *Gnetum*.

The appearance of periclinal divisions in the surface layer during foliar initiation in *Gnetum* is similar to the condition found by Strasburger (1872) and by Korody (1937) for the Abietaceae but is contrary to the findings of Cross (1942) in the Taxodiaceae where the surface layer remains discrete during leaf initiation as is also the case in *Ephedra* (Gifford, 1943). Considerable variation in this characteristic also occurs in the angiosperms; a discrete outer tunica layer is common to all investigated dicotyledons

(Foster, 1949) and to certain monocotyledons (Sass, 1944) but in others, particularly the Gramineae, periclinal divisions make a substantial contribution to the developing leaf (Sharman, 1945).

Morphologists, since the discovery of the zoned apex in *Ginkgo* (Foster, 1938), have placed increasing emphasis on zonal structure not only in the gymnospermous but in the angiospermous apex as well. Philipson (1949) has cited evidence from his work and from that of others in support of the contention that the angiospermous apex exhibits zonation which has been largely overlooked because of adherence to the tunica-corpus concept in recent studies. He is of the opinion ". . . that a central initial zone above a meristem with smaller denser cells arranged in more or less definite rows (file meristem) and surrounded by a peripheral zone of cells with very dense contents is typical of the angiospermous as well as the gymnospermous shoot apex." A central region of permanently embryonic tissue extending from the surface of the apex to varying depths within the shoot and from which the remainder of the apex is ultimately derived, is a feature apparently possessed by all vascular plants. Two fundamental patterns occur at the surface of this region of initiation: 1) a conspicuous apical cell which cuts off segments in a regular sequence from its lateral faces as is seen in *Equisetum*, many species of *Selaginella* and the leptosporangiate ferns; and 2) a group of superficial initials. These divide: a) both periclinically and anticlinally as in *Lycopodium*, certain species of *Selaginella*; eusporangiate ferns and practically all gymnosperms or b) anticlinally as in the majority of the angiosperms. *Gnetum* is clearly at the angiosperm level in this respect.

If the sub-apical portion of the initiation zone as Philipson (1949) suggests is homologous in both gymnosperms and angiosperms, and certainly position and contribution to the remainder of the apex supports this hypothesis, then the various physiological and structural expressions in this zone may be properly regarded as a matter of degree and detail. This hypothesis has the advantage of emphasizing essential similarity although further research will be necessary before apical growth and structure can be classified in definite categories. The characteristics, however, for several rather distinct types are emerging from recent studies. The cycad type of initiation zone, with active sub-apical initials producing a conspicuous and cyto-histologically distinct zone of central mother cells, is well established even though considerable variation exists in the structure of the central mother cell zone as seen in *Zamia*, *Cycas* and *Microcycas* (Johnson, 1939; Foster, 1939, 1940, 1943). *Ginkgo* represents a second type, which while similar to the cycad type differs aside from the size of the various zones in that the surface layer contributes directly to the central mother cell zone and the central mother cell zone does not show the vertical stratification seen in some of the cycads, as for example *Microcycas*. The initiation zone in *Sequoia* and *Pseudotsuga*, as described by Sterling (1945, 1946) resembles that in cycads.

Likewise the combination of sub-apical initials and central mother cell zone in *Gnetum* is more similar to the condition in cycads than to other described apices. A third type of structure is of frequent occurrence among the Coniferales; it consists of a relatively small zone of more or less irregularly arranged sub-apical cells which give rise to the flanking layers and a central core of pith (Koch, 1891, Korody, 1937). The only recorded approach in the conifers to the central mother cell zone of *Ginkgo* and the cycads is in *Sequoia* and *Pseudotsuga*. Turning to the other two members of the Gnetales we find, according to Bower (1881), that the cells of the apex of *Welwitschia* present no point of special interest but resemble the condition in the Coniferales; while Gifford (1943) shows a small zone of irregularly arranged sub-apical initials in *Ephedra altissima* resembling that in the coniferales.

A central zone of mother cells with varying cyto-histological characteristics has been described frequently for angiosperms. Some of the categories realized to date are: 1) central cells of the corpus somewhat larger, more vacuolate and dividing less frequently than the cells of the flanks; this type has been reported in a number of papers cited in a review article by Philipson (1947a); 2) a central zone "of a mass of small granular cells as in *Succisa pratensis* (Philipson 1947b); 3) a zone at the apex of the corpus composed of blocks of cells enclosed within the original wall of the mother cells as in *Lupinus alba* (Ball, 1949) or 4) a cup-shaped central zone of enlarged, vacuolate cells with lightly staining nuclei and little mitotic activity as demonstrated by Millington and Gunckel (1950) in *Liriodendron*. Unlike other plants possessing a central zone, that in *Liriodendron* includes both a two- or three-layered tunica as well as the tip of the corpus. The complete histological picture of large cell size, high degree of vacuolation, irregular cell arrangement, groups of daughter cells enclosed within the wall of a single mother cell, wall thickenings—especially where several cells make contact, and prominent pit fields as seen in *Ginkgo* and the cycads seem not to have been duplicated in angiosperms. The structure of the central mother cell zone in *Gnetum* more closely resembles that in the cycads and *Ginkgo* than in any described angiosperm.

The pronounced activity in the rib meristem of *Gnetum* is similar to that in the long shoots of *Ginkgo* (Foster 1938, Gunckel and Wetmore 1946). The latter workers have shown that there is a positive correlation between the duration of activity in the rib meristem and its derivatives and the short shoot and long shoot patterns of growth. Little activity results in short shoots while activity over a long period gives long shoots. The method of increasing the number of files of rib meristem in *Gnetum* differs from that in *Ginkgo*. In both, files may be established by initials arising from anticlinal divisions at any level in the meristem but in *Gnetum* the rib meristem, which originated in the periphery of the central mother cell zone, is obliterated by anti-

clinal divisions only to be reconstituted with an increased number of files in the first internode.

The flanking layers of the apices of vascular plants consist of relatively small, actively dividing cells from which leaf primordia, cortex, and the vascular tissues take their origin. Periclinal divisions are frequent in the pteridophytes and exceedingly so in the cycads and *Ginkgo* where long files of cells are built up at right angles to the surface. This is as constant a feature in the cycads as is the presence of a central mother cell zone. Periclinal divisions have been largely eliminated in the Coniferales, Gnetales and the Angiosperms.

SUMMARY

1. The structure of the shoot apex of *Gnetum gnemon* is described. The superficial layer has attained the status of a true tunica. It is discrete at the summit but contributes to the inner tissues of the foliar buttress and rarely may show periclinal divisions on the flanks.

2. The summit of the corpus is occupied by a group of initials which give rise to a flanking mantle of actively dividing cells and to a zone of underlying central mother cells. The flanking layers divide by both periclinal and anticlinal divisions. Division is in all planes in the central mother cell zone but is less frequent than in the flanking layers. The cells often appear in blocks, may be highly vacuolate and larger than their neighbors. A rib meristem is derived from the periphery of the central mother cell zone. During the initiation and early growth of the leaf primordia anticlinal divisions disrupt the rib meristem but it is reconstituted later in the first internode.

3. A comparison is made between the structure of the apex of *Gnetum gnemon* and that of other gymnosperms and angiosperms. It is concluded that *Gnetum* has attained the angiospermous level in the possession of a distinct tunica and corpus but that a gymnospermous trend is reflected in the organization of the central mother cell zone recalling the condition seen in the cycads and *Ginkgo*.

DEPARTMENT OF BOTANY, RUTGERS UNIVERSITY
NEW BRUNSWICK, N. J.

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AEGLOPSIS CHEVALIERI IN LIBERIA[1,2]

J. T. BALDWIN, JR. AND BERNICE M. SPEESE

Aeglopsis Swingle is a genus of two species in the Rutaceae, Subfamily Aurantoideae, Tribe Citreae, Subtribe Balsamocitrinae. This subtribe encompasses thirteen species in seven genera, which with one exception (*Swinglea glutinosa*) have hard-shelled citroid fruits (Swingle, 1943). Three of the genera are African; the others are in the Philippines, Java, and Southeast Asia.

Swingle (1912) established *Aeglopsis* for a small tree from 1–4 meters in height: *A. Chevalieri*. The type, well-illustrated in the original description, is *Chevalier 17940*, May 1–5, 1907, Sassandra, Ivory Coast, on sandy soil near the sea and just back of a mangrove swamp. The paratype is *Pogeguin 180*, 1895, Tabou, Ivory Coast, within two hundred meters of the sea; seedlings from this locality were grown in the Jardin des Plantes at Paris. These are the only collections for the species cited either by Hutchinson and Dalziel (1928) in the *Flora of West Tropical Africa* or by Swingle (1943) in his monograph on *Citrus* and its wild relatives. But the latter authority states that seedlings [probably originating from the Tabou locality] have been grown at various places in the United States, and specimens of such cultivated plants are in the Herbarium of the U. S. National Arboretum.

On February 28, 1950, in high bush near Bobei, Sanokwele District, Liberia, the senior author found a twenty-foot tree of *A. Chevalieri: Baldwin 14182*, (fig. 1). Copies of the photograph reproduced as figure 1 have been deposited in the herbaria of the U. S. National Arboretum, the Smithsonian Institution, and Kew Gardens. Specimens of *Baldwin 14182* are in the National Arboretum and will be distributed elsewhere. The tree bore only six fruits. Though without flowers the specimens were easily referred to *Aeglopsis*, this being the only rutaceous genus in Hutchinson and Dalziel's *Flora* with simple leaves. Dr. W. T. Swingle later checked this determination. The fruit shown in figure 1 has five cells filled with shining, glabrous seeds that are sticky with somewhat odorous gum.

In spite of having walked extensively throughout Liberia (Baldwin, 1950) and of having traveled widely in West Africa from 1947–1950, the

[1] The collection on which this report is based was made by the senior author in Liberia in 1950 while on a mission in West Africa for the Division of Plant Exploration and Introduction, U. S. Department of Agriculture.

[2] Illustrations published with the assistance of the Lucien M. Underwood Memorial Fund.

Fig. 1. Herbarium specimen of *Aeglopsis Chevalieri* from Bobei, Liberia. Photo. Robert L. Taylor.

senior author has observed only this single tree of *Aeglopsis*. But the Mano natives in Liberia recognized the tree as being "wild orange" and reported it not to be infrequent, so perhaps *Aeglopsis* is not so rare as the limited collections would imply. The Bobei collection is significant in several respects: the tree is considerably bigger than any specimens discussed by Swingle for the species; occurrence one hundred fifty miles inland establishes the fact that the natural habitat is not restricted to the proximity of the sea; the locality becomes the northwestern-most station known for this African complex of genera.

A. Chevalieri has served effectively as a rootstock for closely related genera, but no graft-relations with *Citrus* have been successfully executed (Swingle, 1943; Swingle, Robinson, and May, 1929). Nor have attempts to hybridize this species with *Citrus* representatives succeeded. But genetic strains of *A. Chevalieri* in cultivation have perhaps been limited to those derived from a single tree in nature. For experimental reasons, therefore, seedlings of *Baldwin 14182* have been established at the Firestone Plantation, Harbel, Liberia, at the College of William and Mary, and at certain agricultural stations in the United States.

A. Chevalieri has an n-number of 9 (Longley, 1925) and a $2n$-number of 18 chromosomes (fig. 2).

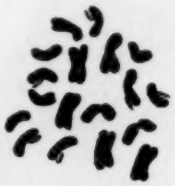

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FIG. 2. Chromosomes of *Aeglopsis Chevalieri* from propio-carmine leaf smear: $2n = 18$. Magnification X2200.

The second species of the genus, *A. Eggelingii* M. R. F. Taylor, is known from Uganda and the Anglo-Egyptian Sudan. Eggeling (1940) stated that this is a spiny bush or tree to twenty feet high with "fruit yellow-green, woody, pear-shaped, up to 3 in. in diam.; seeds surrounded by a strongly aromatic balsamic resin." It would appear that the two species are very closely related.

SUMMARY

A collection of *Aeglopsis Chevalieri* from the hinterland of Liberia is reported. Previously the species was known from two seacoast stations in the Ivory Coast. Seedlings have been established in cultivation. A $2n$-number of 18 has been determined; this corroborates an n-number-of-9 report in the literature.

The Liberian material affords evidence that *A. Chevalieri* has very close affinities to *A. Eggelingii*, which is the only other species in the genus.

COLLEGE OF WILLIAM AND MARY
WILLIAMSBURG, VIRGINIA

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BULLETIN OF THE TORREY BOTANICAL CLUB
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A SURVEY OF TISSUES THAT REDUCE 2,3,5-TRIPHENYL-TETRAZOLIUM CHLORIDE IN VASCULAR PLANTS[1]

L. W. ROBERTS

Tetrazolium differs from the majority of redox indicators since, in the reduced state, it forms an insoluble formazan and the reaction is, therefore, irreversible. It is easily visible in minute quantities and, consequently, the reaction is very sensitive. Tetrazolium readily penetrates the majority of plant tissues, and it is not adsorbed. The formazan is insoluble and neither diffuses from the cell in which it was formed, nor oxidizes back to a colorless state on standing. In the last few years tetrazolium salts have found a wide application as indicators of reducing activity in plant and animal tissues. A preliminary survey of the use of 2,3,5-triphenyltetrazolium chloride as a dye for vital tissues was conducted by Mattson, Jensen, and Dutcher (1947). Waugh (1948) surveyed the reaction of 2,3,5-triphenyltetrazolium chloride in stem tissues. The present investigation was primarily concerned with a survey of tissues that bring about reduction of tetrazolium to formazan. This included a study of apical, lateral, and intercalary meristems in the stems and roots of representatives of several plant families. The inner and outer cortex of roots and stems brought about reduction of the indicator, and some unique indicator patterns of this type were observed in plants with tetrarch root systems. Wound reactions were also tested with 2,3,5-triphenyltetrazolium chloride.

Material and methods. In nearly all cases freehand sections of fresh material were used. The fresh material was placed in a small slit in a piece of pith which had been previously soaked a few minutes in water. The freehand sections were cut with a razor. The sections and pith matrix were easily removed from the blade to a microscope slide by a stream of water from a small pipette. The pith matrix and sections were then separated with forceps and dissecting needle. The indicator (2,3,5-triphenyltetrazolium chloride) was applied dropwise to the sections. A cover glass was added and the preparation allowed to stand for several minutes until reduction had occurred.

In the case of seed embryos, root tips, and other gross preparations, a glass spot plate was used as a receptacle for the test material. It was found advantageous to test weak and sluggish reactions in a desiccator attached to a vacuum line to aid penetration of the tissues by the test solution.

[1] Illustrations published with the assistance of the Lucien M. Underwood Memorial Fund.

The freezing microtome proved inapplicable for sectioning fresh material since the characteristic reduction was either greatly changed or completely absent after freezing. If a sterol wax matrix (Carbowax 4000) was used instead of water, the reduction was more nearly normal.

A preliminary survey was made of various dicotyledons, monocotyledons, and ferns: *Acer Negundo* L.,[2] *Acer saccharinum* L., *Ailanthus altissima* (Mill.) Swingle, *Aesculus glabra* Willd., *Asimina triloba* (L.) Dunal, *Citrus aurantifolia* (L.) Swingle, *C. limonia* Osbeck, *C. sinensis* Osbeck, *Cucurbita maxima* Duchesne, *Cyperus* spp., *Equisetum arvense* L., *Euonymus atropurpureus* Jacq., *Forsythia* spp., *Iris* spp., *Kalanchoe* spp., *Lilium tigrinum* Ker., *Lycopersicum esculentum* Mill., *Malus pumila* Mill., *Morus rubra* L., *Nerium oleander* L., *Pelargonium* spp., *Peperomia* spp., *Phaseolus vulgaris* L., *Platanus occidentalis* L., *Polystichum Lonchitis* (L.) Roth., *Prunus Persica* (L.) Batsch, *Pteris aquilina* L., *Quercus alba* L., *Salix interior* Rowlee, *Sambucus canadensis* L., *Sansevieria* spp., *Smilax hispida* Muhl., *Tradescantia virginiana* L., *Triosteum perfoliatum* L., and *Ulmus americana* L.

The seed embryos tested included: *Abies balsamea* Mill., *Chamaerops humilis* L., *Cucurbita maxima* Duchesne, *Cupressus arizonica* Greene, *Juniperus communis* L., *J. horizontalis* Moench, *Phaseolus vulgaris* L., *Phoenix dactylifera* L., *Picea engelmanii* Darry, *P. monophylla* Torr. and Frem., *P. Pinaster* Ait., *Pseudosuga taxifolia* (Poir.) Britt., *Ricinus communis* L., *Sequoia gigantea* (Lindl.) Decne., *Taxus baccata* L., *Thuja occidentalis* L., *Trachycarpus Fortunei* H. Wendl., *Tsuga canadensis* (L.) Carr., and *Zea Mays* L.

The root tips tested included: *Allium cepa* L., *Capsicum annuum* L., *Cucurbita maxima* Duchesne, *Phaseolus vulgaris* L., *Raphanus sativus* L., *Ricinus communis* L., and *Vicia sativa* L.

Results. Reduction zones in stem tissues of vascular plants. The tetrazolium reaction was observed in apical, lateral, and intercalary meristems. Sluggish lateral and intercalary meristems, and those that are influenced by seasonal variation, gave a much weaker reaction. Strong reducing sites were found in the apical meristems of terminal and lateral buds of deciduous trees. Lateral meristems active in reducing tetrazolium were found in many vascular cambiums and, in some cases, in the cork cambium. Intercalary meristems were also active in meristematic monocotyledonous tissue, and these will be discussed in a separate section. The results of the survey of reduction patterns in stem tissues of vascular plants are given in table 1.

In several of the plants tested certain zones of the cortex were found to

[2] Nomenclature for all trees taken from *Check List of the Native and Naturalized Trees of the United States, Including Alaska*, 325 pages, U. S. Forest Service (1944). Gray's *A Handbook of the Flowering Plants and Ferns*, Seventh Ed. American Book Co., New York, 1908 was used for the nomenclature of the remaining plants.

TABLE 1. *Survey of reduction of tetrazolium in stem tissues of vascular plants.*

Plant tested	Results
Acer Negundo L.	Reduction in outer cortex and vascular cambium.
Acer saccharinum L.	Reduction in inner and outer cortex and vascular cambium.
Ailanthus altissima (Mill.) Swingle	No reduction.
Aesculus glabra Willd.	Reduction in inner cortex, pith parenchyma, and vascular cambium.
Asimina triloba (L.) Dunal	Slow reduction in inner cortex and outer pith.
Citrus aurantifolia (L.) Swingle	No reduction.
C. limonia Osbeck	Reduction in vascular cambium.
C. sinensis Osbeck	No reduction.
Cucurbita maxima Duchesne	Reduction in vascular cambium and distinct rēduction zones in inner and outer cortex.
Cyperus spp.	General reduction in vascular bundles.
Equisetum arvense L.	Reduction in vascular cambium, inner and outer cortex, and phloem.
Euonymus atropurpureus Jacq.	Reduction in xylem parenchyma.
Forsythia spp.	Reduction in vascular cambium and outer cortex.
Iris spp.	Reduction in inner and outer vascular bundles of young leaves.
Kalanchoe spp.	No reduction.
Lilium tigrinum Ker.	Reduction of sclerenchyma in young leaves.
Lycopersicum esculentum Mill.	No reduction.
Malus pumila Mill.	Reduction in vascular cambium and inner cortex.
Morus rubra L.	Reduction in vascular cambium.
Nerium oleander L.	Reduction in inner and outer cortex.
Pelargonium spp.	No reduction.
Peperomia spp.	No reduction.
Phascolus vulgaris L.	Reduction in vascular cambium and xylem parenchyma.
Platanus occidentalis L.	Reduction in vascular cambium.
Polystichum Lonchitis (L.) Roth.	No reduction.
Prunus Persica (L.) Batsch	No reduction.
Pteris aquilina L.	No reduction.
Quercus alba L.	No reduction.
Salix interior Rowlee	Reduction in vascular cambium and phloem.
Sambucus canadensis L.	No reduction.
Sansevieria spp.	No reduction.
Smilax hispida Muhl.	Reduction in inner cortex.
Tradescantia virginiana L.	Reduction in inner and outer cortex.
Triosteum perfoliatum L.	No reduction.
Ulmus americana L.	Reduction in vascular cambium.

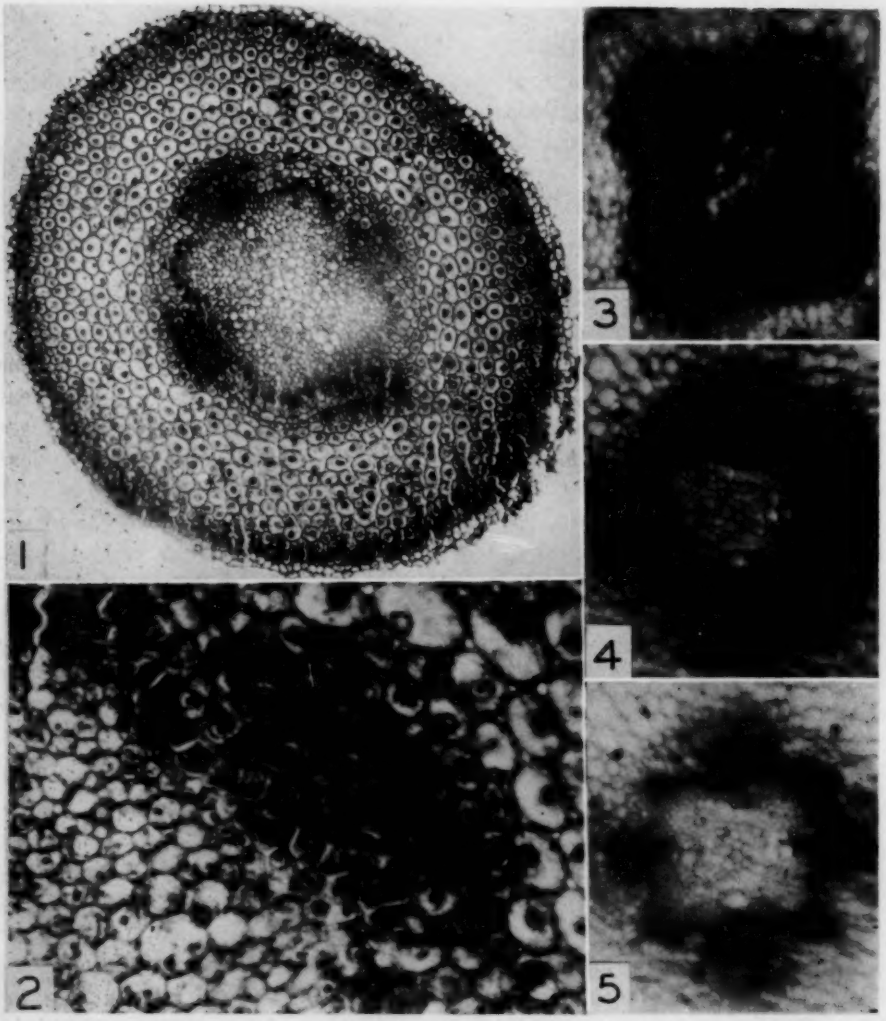
be sites of reducing activity, and these tissues did not correspond with the location of any previously reported meristematic tissues. Since these zones are physiologically separated from neighboring tissues by a greater reducing power, there is the appearance of a cortical meristem. Williams (1948) has reported a continued cell division in this region. The cortical cells in a number of plants (*Cornus spp.*, *Acer Negundo* L.) have been shown to retain their meristematic activity. The phellogen arises in most plants from re-

peated divisions of outer cortical cells. Secondary thickening in certain monocotyledons may be associated with the activation of cortical tissues resulting in the formation of secondary bundles and interfascicular parenchyma. Wound meristems may be initiated in cortical tissues. Apparently the innermost and outermost cortical cells are more active in division than those of the mid-cortex.

Monocotyledonous reduction patterns. Strong reduction sites were found in the nodes and meristems of leaves in monocots. A difference in the staining pattern can be detected at the beginning of tissue differentiation in young leaves. The vascular strands and tissue at the inner and outer peripheries of the leaf continue to reduce the tetrazolium. As tissue differentiation continues in more mature tissue, the vascular cambium and the phloem retain their reducing power. In a whorl of leaves, the youngest leaf in the center gave the strongest reduction and the outermost leaves give a weaker reduction. A concise gradient was observed from young to old leaf tissue.

Root tip reduction patterns. Actively growing root tips of all the plants surveyed in the work reported here showed some degree of reducing activity. The strongest reduction was in the zone of cell division, with continuing reduction of progressively less intensity, from the apex back, in the outermost and innermost regions of the cortex. The inner and outer cortical regions of reduction observed here are analogous to those found in cortical tissues of stems. In some cases, the cells in these regions were smaller and resembled those of meristematic tissue rather than mid-cortical cells.

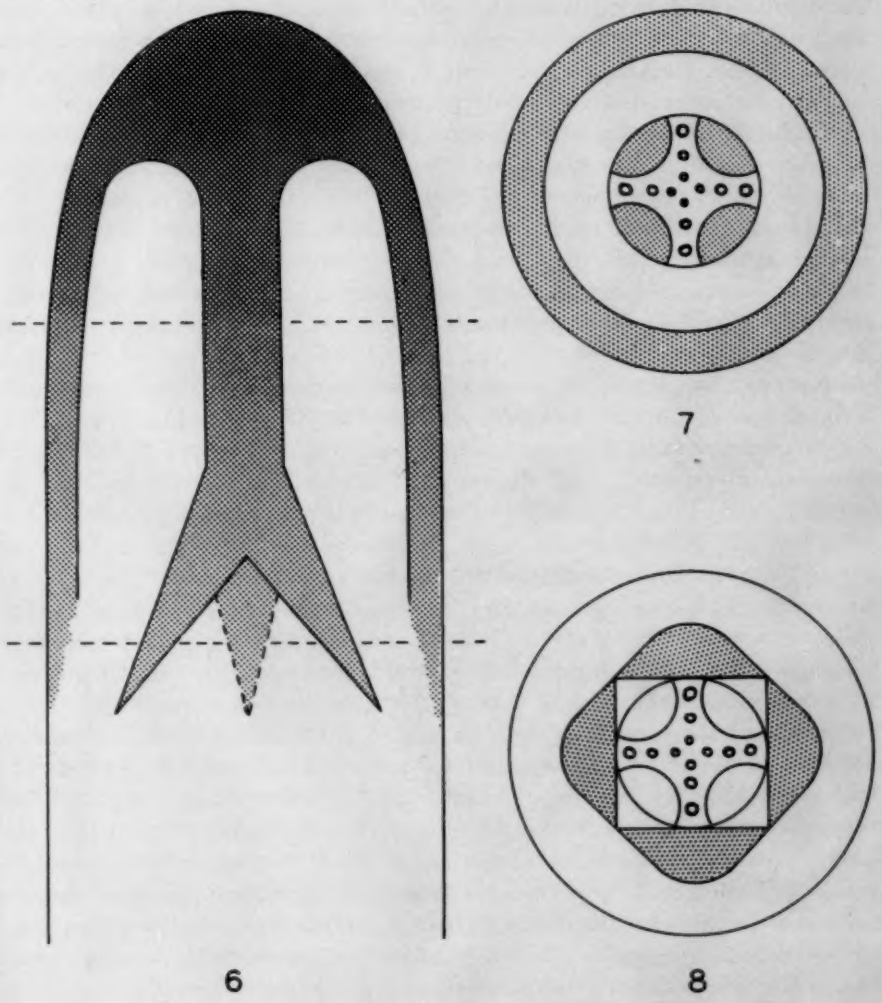
Tetrarch root patterns. The reduction patterns of the root tips of *Phaseolus vulgaris* L. and *Ricinus communis* L., both of which possess tetrarch root systems, were examined. Since tetrazolium is reduced by meristematic tissues, the pericycle and cortical cells which give rise to secondary roots should reduce the indicator preferentially. This reduction would be expected during cell division prior to the emergence of secondary roots in these regions. This hypothesis was borne out experimentally in sections which showed reduction of tetrazolium in a tetrapolar pattern. Reduction of the indicator by the pericycle at the sites of secondary root origin was also observed in *Zea Mays* L. It is important to note that all of these reduction patterns were observed in sections taken near the apex in a stage of development which would preclude the possibility of secondary root formation. The reduction observed was indicative of the sites for root origin, but no evidence for secondary roots was observed in the stages examined. The reduction has therefore offered a means of physiological differentiation of tissues, and indicates the possibility of using tetrazolium in detecting the high metabolic activity of certain tissues. Certain cortical regions, although anatomically the same as contiguous tissue, showed a great difference in metabolic activity as indicated by tetrazolium reduction.



FIGS. 1–5. Photomicrographs of cross sections of root tips of *Phaseolus vulgaris*.

The characteristic staining pattern, observed in root tips possessing tetrarch steles, is illustrated in figures 1–5. A section of *Phaseolus vulgaris* L., stained with safrarin-fast green, is shown in figure 1. It is evident from this section that the cells which reduce tetrazolium also absorb more basic (safrarin) and acidic (fast green) dyes than other cells in the root tip. Figure 2 shows an enlargement of the cortical region opposite a xylem pole (450×). Figures 3–5 are photomicrographs of freehand sections of freshly cut root tips of *Phaseolus vulgaris* L. subsequently stained with tetrazolium. These sections show the typical tetrapolar reduction pattern associated with

roots possessing tetrarch steles. Root tips of *Phaseolus vulgaris* L. reduced the indicator very readily, and this typical reduction pattern was observed within a few minutes. The staining became progressively more intense, and within 10 minutes a general reduction was observed throughout the section. For this reason, it was very difficult to obtain photomicrographs that would illustrate this pattern.



FIGS. 6–8. Diagram of reduction pattern of a root tip of *Phaseolus vulgaris*.

The reduction pattern of a root tip of *Phaseolus vulgaris* L. is diagrammatically shown in figures 6–8. Figure 6, a longitudinal section of a root tip, depicts the gradual decline of the apical meristem. The meristem declines,

from the apex back, in reducing intensity and in area of reduction. The inner and outer cortex are "sustaining" meristematic regions. The outer cortical reduction area becomes progressively weaker and finally disappears. The inner cortical reduction area is centered in the protophloem. These inner and outer cortical reduction areas are shown, in cross section, in figure 7. The inner cortical reduction area, centered in the protophloem, gradually declines as the root tip matures. Cortical areas, opposite the xylem polar groups, now become progressively stronger in their reducing intensity. These cortical areas, diagrammed in figure 8, show the typical tetrapolar reduction pattern associated with roots possessing tetrarch steles.

Reduction zones in seed embryos. The ability of viable seed tissues to reduce tetrazolium serves as a basis for predicting the results of seed germinability tests, as reported by Lakon (1942), Mattson, Jensen, and Dutcher (1947), Porter, Durrell and Romm (1947), and Cottrell (1948). These workers agreed that only those embryos are germinable in which the plumule and adjacent tissue bearing root primordia are stained. Generally, the entire embryo will stain, with the plumule and the radicle the sites of the most intense reactions.

A survey was conducted on members of the *Phenicaceae* and representatives of several families in the *Gymnospermae*. The seeds tested included: *Abies balsamea* Mill., *Chamaerops humilis* L., *Cupressus arizonica* Greene, *Juniperus communis* L. var. *montana* Ait., *J. horizontalis* Moench, *Phoenix dactylifera* L., *Picea engelmanii* Engelm., *P. pungens* Engelm., *Pinus Jeffreyi* Balfour, *P. lambertiana* Dougl., *P. monophylla* Torr., *P. Pinaster* Ait., *Pseudosuga taxifolia* (Lamb.) Britt., *Sequoia gigantea* (Lindl.) Dec., *Taxus baccata* L., *Thuja occidentalis* L., *Trachycarpus Fortunei* H. Wendl., and *Tsuga canadensis* Carr. The seeds were sectioned, treated with tetrazolium in a spot plate, and examined for signs of reduction at the end of 30 minutes, and again at the end of 6 hours. No attempt was made to isolate the embryos, but the seeds were cut to expose a median section of the embryo. Some of the testae were extremely thick and hard and could be broken only with great difficulty. *Sequoia gigantea* (Lindl.) Dec. seeds were very difficult to section due to their extreme thinness, and embryo dissection would have been virtually impossible. In all the seeds tested, the exposed embryo parts reduced the tetrazolium to the red formazan vigorously. In some cases a faint reaction appeared over the entire cut surface. Such results were found to be due to the presence of microorganisms and were easily detected, since the tetrazolium solution itself was stained red.

Wound reactions. Since meristematic activity is generally associated with the tetrazolium reaction, it was reasoned that an analogous reduction zone might be present in a wound meristem. *Pelargonium spp.* stems were wounded by notching with a razor. Regions of the affected areas were sectioned over time intervals ranging from a few hours to several days. There

was no indication of the presence of a reduction zone in any of the sections tested. Although no reduction was observed in these initial wound meristem stages, reduction would probably occur when the meristem is established. The choice of plant material also plays a major part in wound experiments. As it was shown in an earlier section, cortical tissues in stem sections differ widely in their ability to reduce tetrazolium. In conjunction with a previous survey it was observed that *Allium cepa* L. root tips not only reduced the tetrazolium salt in the characteristic apical zone, but also a narrow band of the red formazan was visible at the opposite or cut end. The formazan was deposited at approximately 0.5 mm. from the point of stimulus. It was further noted that if an *Allium cepa* L. root tip was subdivided into several segments, reduction would occur at the stimulated ends of each segment.

Discussion. Tetrazolium differs from the majority of physiological indicators since, in the reduced state, it forms an insoluble formazan and the reaction is, therefore, irreversible. This is advantageous in plant tissues, because the reaction is easily visible and very sensitive. It is possible to test the penetration of the indicator in plant tissues by reducing tetrazolium with sodium hydrosulfite. Tetrazolium readily penetrates the majority of plant tissues, and it is not adsorbed. The formazan is insoluble and neither diffuses from the cell in which it was formed, nor oxidizes back to a colorless state on standing. The sensitivity of tetrazolium to reducing agents, in general, is indicated by the observation that a water solution of tetrazolium will color a stainless steel spatula an intense red, due to the slight reaction of the metal with water which liberates hydrogen.

In using tetrazolium it was found that staining must be completed, observed, and recorded within 24 hours, since microorganisms may appear and obscure the reaction. Living bacteria, stained by tetrazolium may simulate staining of embryo sections and give apparently erroneous results in germinability tests. Bacterial reduction is readily identified since the tetrazolium solution itself is stained red, and the improperly stained sections are readily distinguishable from those showing normal coloration. For example, contaminated sections may be covered with minute red spots, indicating the presence of bacterial colonies.

Plant meristems, regardless of their positions in the plant, are strong reducing zones for tetrazolium. It is possible to use this indicator in detecting the high reducing activity of certain tissues. Examples of this were found in stem sections. Certain cortical regions, although anatomically the same as contiguous tissue, showed a great difference in reduction of tetrazolium. This difference was also observed in root tips possessing tetrarch steles; such root tips reduced tetrazolium in tissue opposite the xylem rays which later divided to give rise to secondary roots. However, reduction was observed at a stage prior to pericyclic division. If it is possible to use tetrazolium in detecting physiological differences in the same tissue, wounded

tissues should show a zone of higher reducing activity than contiguous unstimulated tissue, but experimental attempts to show this were unsuccessful. However, it was observed that *Allium cepa* L. root tips not only reduced tetrazolium in the characteristic apical zone, but also a narrow band of formazan was visible at the opposite or cut end. The formazan was deposited approximately 0.5 mm. from the point of stimulus. Furthermore, if the root tip is subdivided into several segments, this narrow band of formazan is observed in the same position on each of the stimulated or cut ends. This observation suggests the origin of a wound meristem.

SUMMARY

Observations were made on the reduction zones in stem tissues of vascular plants. Apical meristems of terminal and lateral buds of deciduous trees were reducing zones for tetrazolium. Lateral meristems reducing tetrazolium included cork cambium, vascular cambium, and inner and outer cortical regions. Intercalary meristems, found at the bases of the leaves of many monocotyledons, reduced tetrazolium. Reduction zones in root tissues were observed. Actively growing root tips of all the plants surveyed showed some degree of reducing activity. Inner and outer cortical reduction regions were observed in meristematic root tip tissue. These zones have not been observed before, and they indicate cortical meristems that have not been described to date. Plants possessing a tetrarch root system reduced tetrazolium in a tetrapolar pattern which corresponded to the pattern of secondary root formation. This reduction, in the pericycle and cortical cells, was in a stage of development which would preclude the possibility of secondary root formation. Reduction zones in seed embryos were observed. Generally the entire embryo was stained, with the plumule and the radicle the sites of the most intense reactions. In some cases spurious results were observed as a result of tetrazolium reduction by microorganisms. Experimental wounding of plant tissues did not reveal, by tetrazolium reduction, the presence of a wound meristem. However, it was noted that if an *Allium cepa* L. root tip was subdivided into several segments, reduction would occur at the stimulated ends of each segment.

DEPARTMENT OF BOTANY, UNIVERSITY OF MISSOURI,
COLUMBIA, MISSOURI.

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UPTAKE AND ACCUMULATION OF PHOSPHORUS^{32} BY NORMAL AND GALL TISSUES

TSUNG-HSUN TSAO AND W. GORDON WHALEY

It has been reported (Erf & Lawrence 1941) that animal tumor tissues show a higher and more rapid accumulation of administered radiophosphorus than normal tissues. Certain gall tissues are, in some respects, similar to animal tumor tissues (White & Braun 1943). No reports have yet been made on the accumulation of radiophosphorus by gall tissue in plants.

Potted greenhouse plants of *Bryophyllum calycinum* L. were induced to form galls by being inoculated by repeated pricking with a needle previously dipped in a 24-hour old suspension of the crown gall organism (*Agrobacterium tumefaciens* (Smith and Townsend) Conn strain, A_1-Riker). Inoculations were made on both the midribs of the leaves and on the stem internodes. The P^{32} absorption experiments were begun five weeks after inoculation, when the galls had reached a diameter of about 2 mm. Since Bryophyllum has opposite leaves, pairs of comparable leaves were available for experimental purposes.

Approximately 32 microcuries of phosphorus^{32} in the form of orthophosphoric acid were added to the nutrient solution [Shive's four-salt solution (Wolkoff 1918)]. The activity level of the P^{32} varied somewhat from one experiment to another, but no comparisons among experiments were attempted. The radioactive nutrient solution was introduced into the plant tissues through the roots or through the petioles. For root introduction, plants were taken out of the pots, washed free from adhering soil and placed in beakers containing the P^{32} nutrient solution. For petiole introduction pairs of leaves were severed at the bases of the petioles by cutting under water with a sharp razor blade. The ends of the petioles were then placed in P^{32} nutrient solution. Absorption took place under normal laboratory conditions. One 75-watt incandescent lamp was placed two feet from the side of the plant or excised leaves to facilitate absorption.

After an absorption period of known duration, radioactivity assays were made. Both the callus tissue and corresponding normal tissue (on the other leaf of the pair; or at the same level of the stem opposite the gall) were cut off, weighed and ground in a mortar. A known amount of the resulting ground tissue was transferred to a graduated tube and made up to a standard volume. Appropriate aliquots were pipetted into aluminum pans and dried slowly on a hot plate. In some experiments, the weighed fresh tissues were ground directly in the aluminum pans with a flat-end glass rod. Radio-

activity was determined by means of a Geiger-Muller radioactivity counter. The values were corrected for the decay in radioactivity which had occurred during the absorption period. As shown in Figure 1, the leaf gall tissue

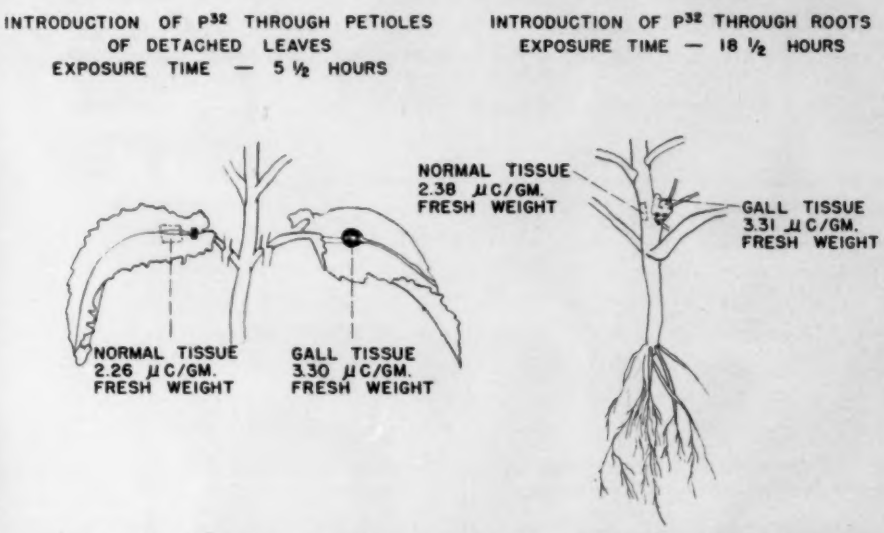
INTRODUCTION OF P32 THROUGH PETIOLES OF DETACHED LEAVES
EXPOSURE TIME — 5 ½ HOURS
INTRODUCTION OF P32 THROUGH ROOTS
EXPOSURE TIME — 18 ½ HOURS
NORMAL TISSUE 2.38 μC/GM. FRESH WEIGHT
GALL TISSUE 3.31 μC/GM. FRESH WEIGHT
NORMAL TISSUE 2.26 μC/GM. FRESH WEIGHT
GALL TISSUE 3.30 μC/GM. FRESH WEIGHT

FIG. 1. Radioactivity per gram of tissue, fresh weight, in galls and comparable normal tissue of the leaves and stems of *Bryophyllum calycinum* after absorption of P^{32}.

accumulated about 40 percent more P^{32} per unit of fresh weight than the corresponding normal tissue of the opposite leaf. Less P^{32} was accumulated in galls per given unit of time when the P^{32} was introduced through the roots than when it was introduced through the cut petioles. The stem gall tissue also showed approximately a 40 percent increase in P^{32} uptake over the normal tissue at the same stem level. The roots which developed on the galls were cut off and not included in the radioactivity assays.

A separate experiment where absorption was allowed to take place through the petioles of normal leaves for a four and a half hour period showed that on a tissue weight basis the midrib tissue had accumulated five times as much P^{32} as the rest of the leaf blade. Further, the young leaves (the uppermost pair) had counts four to five times higher than the older leaves (the fifth or the lowest pair), on a unit fresh weight basis after an 18-hour absorption through the roots. When P^{32} was introduced through the petioles of leaves with galls, the radioactivity of the portion of midrib below the gall (morphologically basal) relative to the portion above the gall was 1.5 to 1.0 on a unit fresh weight basis. Apparently the gall acted to absorb and hold the P^{32} in such a manner as to result in lower radioactivity counts in the midrib between the gall and the apex of the leaf.

Three-week old tomato plants (*Lycopersicon esculentum* Mill.) grown

in the greenhouse were also induced to form galls by the method described above. These galls were collected and assayed for total phosphorus by the Fiske-SubbaRow (1925) method. Similarly normal leaf and stem tissues and insect galls on leaves of *Celtis laevigata* K. Koch growing on the campus of the University of Texas were collected and assayed for total phosphorus. The results are shown in Table 1. The data show clearly that the phosphorus

TABLE 1. *Accumulation of phosphorus in gall tissue in tomato and Celtis.*

Tomato			*Celtis*	
Tissue	Phosphorus Content gamma/mg. dry wt.		Tissue	Phosphorus Content gamma/mg. dry wt.
	Experiment*			
	1	2		
Apex of main stem	3.1	5.1	Leaf	2.1
Old leaves	1.4	3.1	Stem	2.2
Stem	1.2	1.4	Gall tissue	2.9
Root	2.7	2.9		
Gall tissue	5.2	7.6		

* Experiment 1 involved plants bearing five-week old galls; experiment 2 involved plants bearing seven-week old galls.

content of the tomato crown gall tissue is markedly higher than that of normal root, stem and leaf tissue, and that it is even higher than that of normal stem tip tissue. The insect galls also had a higher phosphorus content than the other *Celtis* tissues, although the difference was less marked than in the tomato plants.

Both the radioactivity assays and the total phosphorus assays indicate that more phosphorus is accumulated in plant gall tissues than in adjacent normal tissues. It is suggested that this accumulation of phosphorus may be associated with metabolic differences of the same sort as are found between tumorous and normal tissues of animals.

THE PLANT RESEARCH INSTITUTE, UNIVERSITY OF TEXAS
AND
THE CLAYTON FOUNDATION FOR RESEARCH

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SOOTY STRIPE DISEASE OF BAMBOO IN INDIA

M. J. THIRUMALACHAR AND M. S. PAVGI

While studying the smut flora near the Benares Hindu University campus (India), the writers collected diseased twigs of bamboo (*Bambusa* sp.). The shoots had been killed by a fungus which had formed linear sooty stripes exposing the powdery spores. A careful examination revealed that the fungus was identical with that determined by Padwick as *Ustilago Shiraiana* P. Henn. collected near Gorakhpur, India, and included by Pavgi and Mundkur (1948) in the list of smut fungi published by them. Since the writers had opportunity to examine fresh material, a detailed germination and cultural study of the fungus was carried out, which revealed that it was not a smut but a species of *Papularia* (Fungi Imperfecti). Ramakrishnan T. S. and K. (1948) reported *U. Shiraiana* on *Arundinaria* sp. in South India. Since no specimen of this could be secured, a critcal study of that collection is not included here.

Symptoms. Field observations first gave the indication that the fungus is a wound parasite. Infection always started near wounded stem, working downwards as a die-back. Infection often started on broken young shoots and gradually spread downwards into the main stem. The diseased tissues usually dried up and became greyish-brown. Very soon linear sori were differentiated covering the entire diseased surface. Mature sori are linear, 2 to 8 mm. long, often coalescing to form long striae. They are erumpent and, because of flaking away of the epidermis, powdery black masses of spores are exposed. The spores are loosely held and get dispersed by wind.

Spore germination and artificial culture. The spores are formed within the sori at the ends of loose hyphae. The spore characters are already given by Pavgi and Mundkur. The spores were germinated and stained by the method suggested by Thirumalachar (1940). They readily germinate in water within 30 hours, developing long septate germ tubes (fig. 1). At room temperature (15–17 c.) as much as 100 percent germination was observed within 24 hours. After prolonged incubation in moist chambers, the spores developed extensively-branched hyphae, and there was no indication of promycelium or sporidia.

Germinating spores were transferred aseptically on to potato dextrose agar to secure artificial culture of the fungus. It formed white cottony mycelial growth on the medium. As the culture became old, dark areas were noticed which indicated places of spore development. When incubated at room temperature, abundant spore development was observed after ten days.

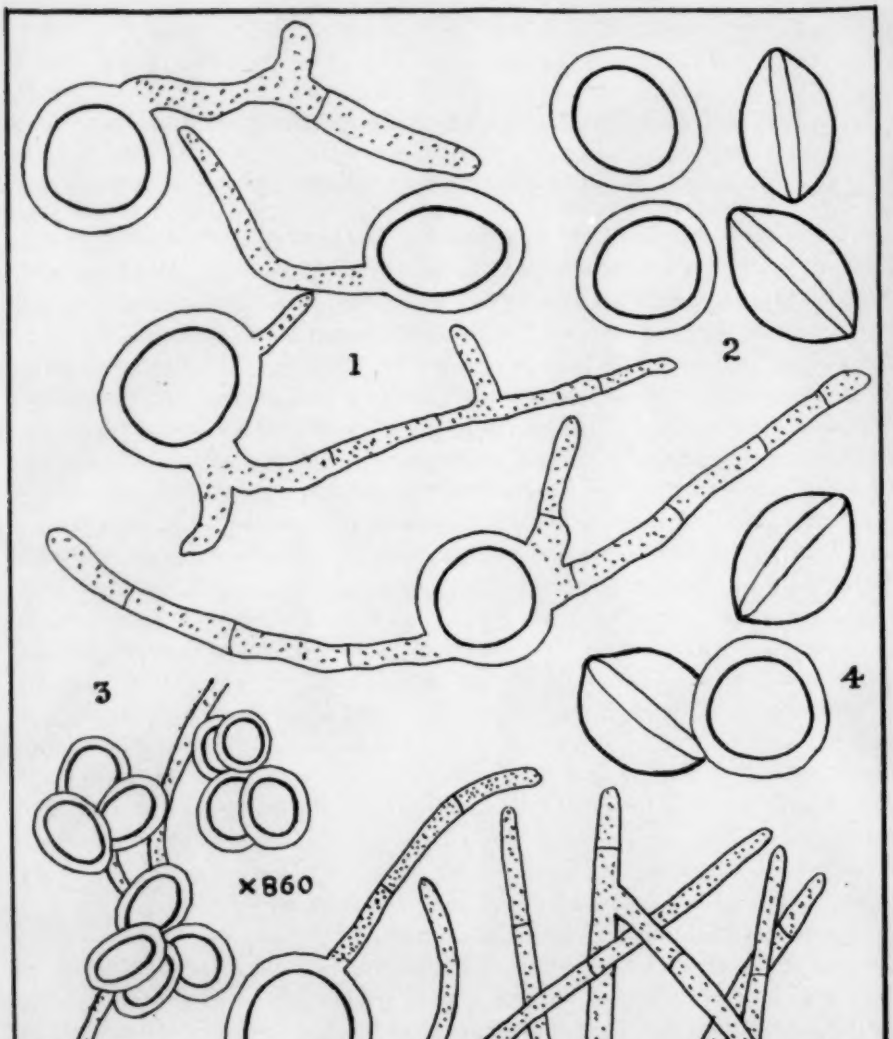

FIG. 1. Germination of the spores produced naturally on the host. ×1920. FIG. 2. Spores showing the flat and ridged sides. ×1920. FIG. 3. Development of spores in artificial culture. ×860. FIG. 4. Spores produced in artificial cultures. ×1920. FIG. 5. Germination of spores from artificial culture. ×1920. FIG. 6. Septate mycelium in culture. ×1920.

Microscopic examination revealed that the hyphae were septate, branched and possessing rich contents (fig. 6). In stained preparations, the hyphae were seen to be multinucleate. The spores were formed in clusters on short stalks (fig. 3). Mature spores are ovate-elliptic to spherical, chocolate-brown, thick-walled, with pale lenticular ridge, measuring 7.5 to 12 μ with a mean of 10.3 μ (fig. 4). Spores produced naturally on the host were somewhat more opaque and measured 7.5–11.5 μ with a mean of 10.5 μ. The spores produced in artificial culture were identical with those produced naturally on the host (fig. 2) except for the slight differences mentioned above. These germinated readily in water, developing long septate germ tubes much as do the naturally produced chlamydospores (fig. 5).

Inoculation experiments. Infection experiments were carried out to determine the nature of pathogenicity. Inoculations were carried out using spores produced in artificial cultures. Young shoots of bamboo were selected from disease-free culms and inoculated by the following methods: (a) The host was bruised by sterile needle before placing the inoculum consisting of spore suspension and mycelial fragments on them; (b) a thin flap of the host tissue was cut with a sterile scalpel and the inoculum was placed beneath; (c) the host was inoculated without any wounding.

In all the cases inoculated portions were wrapped with water-proof paper lined with moist cotton which served as improvised moist chamber. Several controls were set apart for each method of inoculation. Observations revealed that the fungus was slow in establishing itself on the host. Infections were observed 45–50 days in all the fifteen inoculation experiments when the host had been wounded and none at all on those inoculated without wounding and the controls.

Conclusion. The present study has revealed that the fungus is a species of *Papularia* gaining entry into the host through wounds. Earlier determination of this fungus as the bamboo smut *Ustilago Shiraiana* is therefore incorrect. Authentic specimens of *U. Shiraiana* parasitic on *Phyllostachys* in China were obtained through the courtesy of Dr. Lee Ling. Comparative study indicated that the Chinese specimen bears no resemblance to the fungus under study.

As regards the identity, the fungus under study has been placed under *Papularia Arundinis* (Corda) Fr. (= *Coniosporium Arundinis* (Corda) Sacc.) reported on the culms of *Arundo donax* and *A. mauritiana* in Europe, north Africa and North America. The fungus is stated to incite the formation of elongated stripes on the culms, disintegrating the tissues, and producing brownish-black conidia of 8–12 μ diameter with the characteristic lenticular ridge. The same characters are duplicated in our fungus, which has been shown by inoculation experiments to be a wound parasite.

It is of interest to note that *Coniosporium Arundinis* (= *Papularia Arundinis*) is treated as synonym of *Papularia sphaerosperma* (Pers.) v. Höhnel

by Gilman (1945). The latter is a soil fungus with slightly smaller spores measuring 5–9 μ in diameter. It is doubtful whether the phytopathogenic fungus *P. Arundinis* should be treated as a synonym of *P. sphaerosperma*, which in addition to having smaller spores is so far known only as a saprophyte.

BENARES HINDU UNIVERSITY
INDIA

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NOVELTIES IN THE ERIOCAULACEAE AND VERBENACEAE II

HAROLD N. MOLDENKE

Recently there has been sent to me the entire collection of Verbenaceae of Madagascar deposited in the vast herbarium of the Muséum National d'Histoire Naturelle at Paris. Study of this material preparatory to describing this group for Humbert's Flora of Madagascar has revealed a surprisingly large number of novelties, some of which are described on the following pages. To these have been added the descriptions of five tropical American novelties in this family and in the Eriocaulaceae, based on recent collections.

Eriocaulon candidum Moldenke, sp. nov. Herba acaulescens pumila usque ad 9.5 cm. alta; foliis rosulatis graminoideis 3–4 cm. longis, 2–2.5 mm. latis gradatim attenuatis utrinque glabris vel minutissime pulverulentis basin versus perspicue fenestratis; pedunculis 3 stramineis costatis usque ad 9 cm. longis glabris; vaginis latis laxis ca. 3 cm. longis tenuissime membranaceis perspicue fenestratis glabris; capitulis hemisphaericis albis ca. 8 mm. diametro; bracteolis involucratis nigris glabris.

Dwarf acaulescent herb apparently to about 9.5 cm. tall; leaves basal, rosulate, bright green on both surfaces, grass-like, 3–4 cm. long, 2–2.5 mm. wide at the midpoint, gradually attenuate from base to apex, rather blunt at the apex itself, glabrous or microscopically pulverulent on both surfaces, conspicuously fenestrate toward the base, several-nerved; peduncles about 3 per plant, several-costate, stramineous, medium-textured, to 9 cm. long, glabrous, slightly twisted; sheaths broad, loose, about 3 cm. long, very thin-membranous, conspicuously fenestrate, glabrous, the rim obliquely split; heads hemispheric, white, about 8 mm. in diameter; involucral bractlets concave, elliptic, firm-textured, black, 2.5–3 mm. long, about 1.5 mm. wide, acute at the apex, glabrous; receptacular bractlets concave, obovate-spatulate, firm-textured, conspicuous, about 3 mm. long and 1.5 mm. wide, the upper half black, the lower half much paler, acute at the apex, more or less pilosulous toward the apex on the back; staminate florets: sepals 3, black, separate, obovate-spatulate, about 2 mm. long and 0.9 mm. wide, rounded and somewhat cucullate at the apex, densely white-barbellate at the apex on the back; petals 3, white, united into an infundibular tube about 1.5 mm. long, surmounted by 3 separate lobes, of which 2 are much smaller than the third, the small ones about 1 mm. long, the large one 2 mm. long, densely white-pilose on the back with antrorse hairs, with a black gland near the apex of each lobe; stamens 6; anthers oblong, dark-brown; pistillate florets: sepals 3, separate, black, obovate, navicular, about 2 mm. long and 1 mm. wide, rounded and white-barbellate on the back at the apex; petals 3, separate, oblanceolate-spatulate, white, about 2 mm. long and 0.8 mm. wide, rounded and white-barbellate on the back at the apex, with a small black gland almost

at the apex; ovary oblate, about 0.5 mm. long and 0.8 mm. wide, glabrous, deeply 3-sulcate, 3-celled, 3-seeded; style about 0.5 mm. long, glabrous; stigmas 3, about 1 mm. long.

TYPE: *Alarich R. Schultz 700*, in sandy swamps at Tramandaí, Rio Grande do Sul, Brazil, October 30, 1949; Britton Herbarium, New York Botanical Garden.

Leiothrix echinulata Moldenke, sp. nov. Herba acaulescens usque ad 22 cm. alta; foliis caespitosis subcoriaceis graminoideis erectis firmis 3.5–5.5 cm. longis, 2–3 mm. latis, gradatim angustatis, ad basin dense longeque pilosis, caetera glabris nitidis plerumque canaliculato-involutis non fenestratis obscure multinervis; pedunculis 1 vel 2 firme erectis stramineis nitidis ca. 21 cm. longis multicostatis; vaginis membranaceis cylindricis arcte adpressis ca. 3.5 cm. longis glabris; capitulis subglobosis ca. 7 mm. latis echinulatis; bracteolis involucratis numerosissimis ovatis perspicue longeque spinosis vel setosis saepe reflexis glabris.

Acaulescent herb to about 22 cm. tall; leaves cespitose, subcoriaceous, grass-like, erect, stiff, bright green on both surfaces when young, brunnescent in age but still firm and erect, 3.5–5.5 cm. long, 2–3 mm. wide at the midpoint, gradually narrowed from the base to the apex, densely long-pilose at the base, otherwise glabrous and shiny, mostly canaliculate-involute, rather blunt or subacute at the apex, not fenestrate, obscurely many-nerved; peduncles 1 or 2 per plant, firmly erect, stramineous, shiny, about 21 cm. long, many-costulate, very slightly twisted; sheath thin-textured, cylindric, closely appressed, about 3.5 cm. long, glabrous, its apex scarious-split; heads subglobose, about 7 mm. long and wide, echinulate; involucral bractlets very numerous, in many series, spirally disposed, ovate, very conspicuous, 2–2.5 mm. long (of which more than half is a long spinose or setose, often reflexed, caudate acumination), olive-brownish, tough, glabrous; receptacle extremely densely long-villous; receptacular bractlets white, firm, erect, petaloid, few, peripheral, oblong or narrowly elliptic, about 5 mm. long and 1 mm. wide, subacute at the apex, glabrous; staminate florets: sepals 3, whitish-hyaline, erect, separate, elliptic, about 2.5 mm. long and 0.6 mm. wide, acute at the apex, glabrous; petals 3, hyaline, united into an infundibular tube about 1.7 mm. long, surmounted by 3 separate ovate lobes about 2 mm. long, subacute at the apex; pistillate florets: sepals 3, separate, whitish, lanceolate, about 3 mm. long and 0.5 mm. wide, subacute at the apex, glabrous; petals 3, linear, separate, about 5 mm. long and 0.3 mm. wide, densely long-pilose on the lower ¾; ovary oblong, about 0.8 mm. long and 0.5 mm. wide, deeply 3-sulcate, glabrous, 3-celled, 3-ovulate; style gibbous, about 0.4 mm. long, glabrous, its appendages 3, filiform, erect, about 2.1 mm. long.

TYPE: *Paul H. Allen 3199*, at Yapoboda, Vaupés, Colombia, December 10, 1943; Missouri Botanical Garden, St. Louis.

Syngonanthus Alleni Moldenke, sp. nov. Herba usque ad 18 cm. alta; foliis dense rosulatis numerosissimis herbaceis linearibus 3–15 mm. longis obtusis 1-nervatis, subtus dense pubescentibus, supra glabrescentibus; caule singulo atro-brunneo vel nigro glabro 10–11.5 cm. alto ad apicem dense rosulato-bracteato; bracteis linearibus 7–14 mm. longis obtusis divergentibus dense glanduloso-pubescentibus; pedunculis filiformibus numerosissi-

mis umbellatis 2.5–6 cm. longis dense glanduloso-pubescentibus; capitulis obovatis stramineo-griseis 2–4 mm. latis; bracteolis involucri pallide stramineis vel translucentibus ovatis vel oblongo-oblanceolatis acutis vel breviter acuminatis puberulis.

Herb to about 18 cm. tall; leaves densely rosulate, very numerous, apparently closely appressed to the ground, herbaceous, linear, 3–15 mm. long, less than 1 mm. wide throughout, blunt or obtuse at the apex, 1-nerved, densely pubescent on the lower surface, glabrescent above; stem solitary, dark brown or black, issuing from the center of the leaf-rosette, 10–11.5 cm. long, glabrous, terminated by a dense rosette of leaflike bracts and a dense umbel of peduncles; bracts linear, 7–14 mm. long, less than 1 mm. wide, obtuse at the apex, spreading, densely pilose-pubescent with strongly divergent often gland-tipped hairs; peduncles very numerous, filiform, 48–75 per cluster, 2.5–6 cm. long, densely pilose-pubescent with the hairs standing at right angles to the peduncles and gland-tipped; sheaths slender, closely appressed, 2–2.5 cm. long, densely pilose like the peduncles, the blade long, erect; heads obovate, stramineous-grayish, 2–3 mm. long, 2–4 mm. wide; involucral bractlets in several series, pale-stramineous or almost transparent, the outer ones ovate, about 2 mm. long, 1–1.8 mm. wide, acute or short acuminate, minutely puberulent on the outer surface, the inner ones oblong-oblanceolate, about 2.5 mm. long and 1 mm. wide, short-acuminate; receptacle short-villous; receptacular bractlets hyaline, lanceolate, about 3 mm. long and 0.5 mm. wide, long-attenuate at the apex, pilose on the back toward the apex; staminate florets: sepals 3, hyaline, separate to the base, navicular, narrow-elliptic, about 2.5 mm. long and 0.5 mm. wide, attenuate at the apex, pilose-barbellate at the apex on the back; petals 3, united into an infundibular hyaline tube about 2 mm. long, glabrous, 3-lobed at the apex, the lobes involute; pistillate florets: sepals 3, hyaline, separate to the base, navicular, elliptic, about 2 mm. long and 0.7 mm. wide, attenuate at the apex, pilose on the back toward the apex; petals 3, connate at the base and apex, separate at the middle, hyaline, narrow-elliptic or oblong, about 2 mm. long, densely antrorsely pilose on the back; ovary subglobose, about 0.5 mm. long and wide, glabrous, deeply 3-sulcate, 3-celled, 3-ovulate; style and stigmas about 0.5 mm. long in all.

TYPE: *Paul H. Allen 3149* (in whose honor it is named), at Yapoboda, Vaupés, Colombia, December 10, 1943; United States National Herbarium, Washington, D. C.

Callicarpa madagascariensis Moldenke, sp. nov. Frutex 2 m. altus; ramulis gracillimis griseis tetragonis vel subteretibus densissime breviterque pubescentibus, in senectute glabrescentibus foliis oppositis; petiolis gracilibus 3–9 mm. longis densissime flavido-pubescentibus; laminis tenuiter chartaceis lanceolatis vel anguste ellipticis 1.5–5.5 cm. longis, 1–1.6 cm. latis acutis vel breviter acuminatis integris, ad basin obtusis vel rotundatis, supra dense breviterque pubescentibus vel subvelutinis, subtus dense canescenti-flavidulo-tomentellis; inflorescentiis axillaribus terminalibusque parvis cymosis 1–1.5 cm. longis latisque.

Shrub about 2 m. tall; branchlets and twigs very slender, grayish, very obtusely tetragonal or subterete, very densely short-pubescent with flavidous hairs when young, glabrescent in age; nodes not annulate; principal inter-

nodes often much abbreviated on the twigs, 1–8 mm. long, or elongate to 3 cm. on branchlets; leaf-scars comparatively large and elevated, with prominent corky margins; leaves decussate-opposite, crowded at the tips of the twigs; petioles slender, 3–9 mm. long, very densely flavidous-pubescent; blades thin-chartaceous, dark-green above, lighter beneath, lanceolate or narrowly elliptic, 1.5–5.5 cm. long and 1–1.6 cm. wide during anthesis, acute or shortly acuminate at the apex, obtuse or rounded at the base, entire, densely short-pubescent or subvelutinous above, densely tomentellous with canescent-flavidulous hairs beneath; midrib slender, flat above, prominulous beneath; secondaries slender, about 5 per side, arcuate-ascending, flat or obscure above, very slightly prominulous beneath; vein and veinlet reticulation indiscernible above, mostly obscure beneath; inflorescence axillary and terminal, small, cymose, 1–1.5 cm. long and wide, the axillary cymes usually concentrated in the upper axils and appearing as though constituting part of a terminal one, few-flowered, densely short-pubescent with flavidous hairs throughout; peduncles very slender, 2–4 mm. long, flavidous-pubescent; pedicels filiform, 1 mm. long or less, flavidous-pubescent; bractlets linear, 1–2 mm. long, densely flavidous-pubescent; calyx campanulate, about 2.5 mm. long and wide, appressed-pubescent and more or less resinous-granular on the outside, 4-ribbed, its rim shortly 4-dentate; corolla hypocrateriform, its tube about 4 mm. long, lightly puberulent and resinous-granular on the outside above the calyx, its lobes 3–4 mm. long, resinous-granular on the back, lightly pilosulous on the margin and in a median band on the inside; stamens and pistil exserted.

TYPE: *André Seyrig 782*, at an altitude of 750 m., north of Ampandrandava (between Bekily and Tsivory), Madagascar, December 1943; Muséum National d'Histoire Naturelle at Paris.

Clerodendrum alboviolaceum Moldenke, sp. nov. Frutex 5–8 m. altus; ramulis crassis brunneis nitidis obtuse tetragonis parce lenticellatis glabris; foliis oppositis; petiolis crassis 1–2 cm. longis nigrescentibus glabris ad basin ampliatis disciformibusque; laminis coriaceis elliptico-obovatis vel obovatis 15–22 cm. longis, 5.5–9 cm. latis, ad apicem breviter acuminatis, integris, ad basin attenuato-acutis, utrinque glabris; inflorescentiis terminalibus nigrescentibus; cymis 1–3-floris longe stipitatis.

Shrub 5–8 m. tall; branchlets stout, brown, shiny, obtusely tetragonal, rather sparsely lenticellate, glabrous, not twiggy; nodes not annulate; principal internodes 2.5–3.5 cm. long; leaves decussate-opposite; petioles stout, 1–2 cm. long, nigrescent, glabrous, much ampliate and disciform at the base; blades coriaceous, elliptic-obovate or obovate, 15–22 cm. long, 5.5–9 cm. wide, short-acuminate at the apex, entire, attenuate-acute at the base, glabrous on both surfaces, some smaller ones sometimes interspersed with the normal-sized ones; midrib stout, flat above, convex beneath; secondaries slender, 6 or 7 per side, arcuate-ascending, flat above, prominulent beneath, not plainly anastomosing; vein and veinlet reticulation rather fine and abundant, mostly obscure on both surfaces or the larger parts slightly subprominulous beneath; inflorescence terminal, nigrescent throughout; peduncles obsolete or absent, but the 1–3-flowered cymes long-stipitate, the peduncle-like stalks about 5 cm. long, stout, shiny, glabrous; bracts ovate, fleshy, to 7 mm. long and 4 mm. wide, acute, subtending the cyme-branches; bractlets

filiform, subtending the pedicels, caducous; pedicels stout, 1.5–2.5 cm. long, stiffly erect, glabrous shiny; calyx coriaceous, nigrescent, not venose, tubular, 2.5–3.3 cm. long, 12–13 mm. wide, glabrous, its rim 5-lobed, the lobes erect, ovate-triangular, about 5 mm. long and wide; corolla hypocrateriform, its tube violet, narrow-cylindric, 4–5 cm. long, about 1.5 mm. wide above the calyx, ampliate only at the very apex, the limb white, 3–3.5 cm. wide, the 5 lobes 1.2–2 cm. long, obtuse or rounded at the apex; stamens and pistil long-exserted, extending about 3 cm. beyond the mouth of the corolla-tube, violet; fruiting-calyx and fruit not seen.

TYPE: *Henri Perrier de la Bâthie 10271*, on basaltic lava in woods at an altitude of 20 m., Bas Mililam, Madagascar, August 1911; Muséum National d'Histoire Naturelle at Paris.

CLERODENDRUM ARENARIUM var. **macrocalyx** Moldenke, var. nov. A forma typica speciei calyce 2.5–3 cm. longo recedit.

This variety differs from the typical form of the species in having its calyx at time of anthesis 2.5–3 cm. long.

TYPE: *Henri Humbert & G. Cours 17626*, in a shady forest at an altitude of 800–1000 m., Massif de l'Andrangovalo, to the southeast of Lake Alaotra, in the Réserve Naturelle No. 3 (Zakamena), in the basin of the Onibe, Madagascar, end of October 1937; Muséum National d'Histoire Naturelle at Paris. A common name for this shrub is "atamba."

CLERODENDRUM AUCUBIFOLIUM var. **giganteum** Moldenke, var. nov. Haec varietas a forma typicav speciei recedit calyce 4.5–5 cm. longo, ad apicem usque ad 2.5 cm. lato, et tubo corollae usque ad 18 cm. longo gracillimo, lobis ca. 2 cm. longis.

This variety differs from the typical form of the species in having its calyx at time of anthesis 4.5–5 cm. long, flaring to 2.5 cm. wide at the apex, and the corolla-tube to 18 cm. long, very slender except at the infundibular apex, the lobes about 2 cm. long. The fruiting-calyx is deeply 5-lobed, the lobes ovate, about 2 cm. long, erect, attenuate-acute at the apex. The fruit is fleshy, soon splitting the fruiting-calyx, nigrescent.

TYPE: *Henri Perrier de la Bâthie 10229*, in woods on syenite, altitude 200 m., Massif de Monongariva, Sambirano, Madagascar, 1909; Muséum National d'Histoire Naturelle at Paris.

CLERODENDRUM AUCUBIFOLIUM var. **longiflorum** Moldenke, var. nov. A forma typica speciei recedit petiolis pererassis 1.5–3 cm. longis, laminis foliorum firme coriaceis ellipticis vel late ellipticis usque ad 26 cm. longis, 7–12 cm. latis, calyce 3.4–3.8 cm. longo non ampliato ad apicem ca. 1 cm. lato, et tubo corollae gracillimo 11–13 cm. longo, lobis 3–3.5 cm. longis.

This variety differs from the typical form of the species in having its very stout petioles 1.5–3 cm. long, its leaf-blades firmly coriaceous, elliptic or broadly elliptic, to 26 cm. long and 7–12 cm. wide, the calyx at time of anthesis 3.4–3.8 cm. long, not flaring, about 1 cm. wide at the apex, and the corolla-tube very slender except at the infundibular apex, 11–13 cm. long, its lobes 3–3.5 cm. long.

TYPE: *Aubert du Petit-Thouars*, collected somewhere in Madagascar and called "*Volkameria longiflora*" by the collector; Muséum National d'Histoire Naturelle at Paris.

Clerodendrum Boivinii Moldenke, sp. nov. Frutex ca. 2 m. altus sarmentosus; ramis ramulisque griseis prominenter lenticellatis glabratis; sarmentis gracillimis dense albopuberulis; foliis oppositis parvis; petiolis gracillimis 4–12 mm. longis leviter puberulis vel strigillosis; laminis submembranaceis ellipticis vel subovatis 0.8–5 cm. longis, 0.6–3.2 cm. latis integris, ad apicem obtusis vel brevissime acuminatis, ad basin acutis vel subacutis, utrinque glabris, subtus dense punctatis; inflorescentiis axillaribus terminalibusque plerumque laxe multifloris.

Shrub to 2 m. tall, abundantly branched and twiggy; branches medium-slender, gray, prominently lenticellate, subterete, glabrate; branchlets quite slender, very light gray, prominently lenticellate, glabrescent, sometimes ternate; twigs very slender, densely white-puberulent with very short closely appressed antrorse hairs, or on the younger parts the hairs more divergent, sometimes only minutely puberulous; leaves normally decussate-opposite, small, often rather numerous; petioles very slender, 4–12 mm. long, finely puberulent or strigillose; leaf-blades submembranous, rather uniformly grayish-green on both surfaces, elliptic or slightly ovate, 0.8–5 cm. long, 0.6–3.2 cm. wide, obtuse or very shortly acuminate (the point very blunt) at the apex, entire, acute or subacute at the base, glabrous or subglabrate on both surfaces, densely punctate beneath; midrib very slender, mostly flat above, only slightly prominulous beneath; secondaries very slender or filiform, 3–5 per side, mostly obscure on both surfaces or only very slightly subprominulous beneath, arcuate-ascending, not plainly joined; vein and veinlet reticulation indiscernible on both surfaces; inflorescence axillary and terminal, mostly limited to the apex of the twigs, cymose, usually loosely many-flowered, 3–7 cm. long, 4–10 cm. wide; peduncles very slender, 2–2.5 cm. long or occasionally obsolete, more or less puberulent; cyme-branches very slender or subfiliform, mostly elongate and divaricate, puberulent; pedicels filiform, 2–6 mm. long, puberulent or strigillose; calyx campanulate, about 2 mm. long, 1.5–2 mm. wide, minutely puberulent or strigillose, its rim truncate, entire or subentire; corolla white or rose-tinted, hypocrateriform, its tube very narrowly cylindric, usually 9–12 mm. long, rarely to 17 mm. long, minutely pulverulent or glabrate on the outside, its limb 4–7 mm. wide; stamens and pistil exserted about 7 mm. from the corolla-mouth; fruiting-pedicels filiform, to 11 mm. long, glabrescent; fruiting-calyx shallowly cupuliform, about 3 mm. long, 6–7 mm. wide, venose, minutely puberulent or glabrescent, its rim truncate and entire; fruit drupaceous, subglobose, nigrescent, about 7 mm. long and wide, glabrate.

TYPE: *Boivin 2483* (in whose honor it is named), at Port Lewen at the edge of woods along the seashore, Madagascar, April 1849; Muséum National d'Histoire Naturelle at Paris.

Clerodendrum chartaceum Moldenke, sp. nov. Frutex usque ad 3 m. altus; ramulis sarmentisque gracilibus griseis glabris perspicue lenticellatis obtuse tetragonis; foliis oppositis; petiolis crassiusculis 5–9 mm. longis glabris; laminis chartaceis 8–13.7 cm. longis, 3–5.2 cm. latis integris, ad apicem breviter acuminatis, ad basin acutis, ellipticis vel oblanceolatis vel obovatis, utrinque glabris; inflorescentiis terminalibus plerumque 1- vel 2-floris; calyce subcoriaceo tubuloso-obconico 4.3–8.3 cm. longi glabro nitido.

Shrub to 3 m. tall; branchlets and twigs slender, sparse, grayish, glabrous, corky, prominently lenticellate, obtusely tetragonal, the youngest

somewhat compressed and sulcate; nodes not annulate; principal internodes abbreviated, 0.5–4 cm. long; leaves decussate-opposite; petioles stoutish, 5–9 mm. long, glabrous; blades chartaceous, uniformly light green on both surfaces, not at all brunnescent in drying, 8–13.7 cm. long, 3–5.2 cm. wide, short-acuminate at the apex, entire, acute at the base, varying from elliptic to oblanceolate or obovate, glabrous on both surfaces; midrib slender, prominulous above, prominent beneath; secondaries very slender, 7–12 per side, prominulous above, prominent beneath, divergent, only slightly arcuate-ascending, conspicuously but irregularly arcuate-joined near the margins on both surfaces; vein and veinlet reticulation very abundant and prominulous above, only the larger parts prominulous beneath; inflorescence apparently terminal or subterminal, cymose, usually only 1- or 2-flowered, with half of the cyme not developing; peduncles obsolete or about 5 mm. long, sometimes the inflorescence with one developed (and one aborted) main peduncle-like branch to 10 cm. long, somewhat compressed, often somewhat ampliate toward the apex, glabrous, stramineous; pedicels stout, 1–2 cm. long (at time of anthesis), glabrous, stramineous, often curvate; bractlets linear-setaceous; calyx subcoriaceous, tubular-obconic, 4.3–8.3 cm. long, 1.3–2.5 cm. wide at the apex, glabrous, shiny, stramineous, venose (especially on the upper half), its rim deeply 5-lobed, the lobes ovate, erect, firm, 1–1.5 cm. long, mucronate-acute, the 2 or 3 main veins ending at the very tip of the lobe; corolla infundibular, white, suffused with wine-red at the throat, the tube rather broadly cylindric for ¾ of its length, then broadly ampliate in funnelform fashion, 11–15 cm. long, glabrous outside, the limb 5–7.5 cm. wide, the 5 lobes apparently ascending-erect, broadly elliptic or obovate, 2–3 cm. long, 1.5–2.8 cm. wide, obtuse or rounded at the apex, glabrous on both surfaces; stamens and style included by the limb; filaments rose; anthers violet; pistil green; fruiting-calyx and fruit not seen.

TYPE: *Henri Perrier de la Bâthie 10262,* in woods at an altitude of 500 m., Masoala, on the east coast of Madagascar, August 1912; Muséum National d'Histoire Naturelle at Paris.

Clerodendrum dauphinense Moldenke, sp. nov. Planta lignosa ut videtur virgata simplex; ramis graciliusculis brunneis dense breviterque pubescentibus; foliis ternatis; petiolis 2 mm. longis dense breviterque hirsutulo-pubescentibus; laminis ut videtur parvis fragilibus suborbiculari-ovatis vel late ellipticis 1–1.5 cm. longis latisque obtusis integris, ad basin rotundatis, utrinque dense breviterque pubescentibus; inflorescentiis ut videtur parvifloris; calyce campanulato hirsutulo 2–3 mm. longo.

Woody plant, apparently virgate and unbranched; stems rather slender, brownish, densely short-pubescent with widely divergent hairs on the upper parts, less so below; nodes not annulate; principal internodes 1.5–4 cm. long; leaves regularly ternate; petioles very short, about 2 mm. long, densely short-pubescent with hirsutulous hairs; blades apparently very fragile and small, apparently uniformly green on both surfaces, apparently suborbicular-ovate or broadly elliptic, 1–1.5 cm. long and wide, apparently obtuse at the apex, entire, rounded at the base, densely short-pubescent on both surfaces; midrib slender, prominulous beneath, obscure above; secondaries and veinlets apparently obscure or indiscernible on both surfaces; inflorescence apparently very few-flowered; calyx campanulate, 2–3 mm. long, hirsutu-

lous, 1–2 mm. wide, its rim very shortly toothed; corolla white, hypocrateriform, its tube very narrow-cylindric, 5–6 mm. long, glabrous; filaments exserted about 8 mm. from the corolla-mouth.

TYPE: *Raymond Decary 4570,* on granitic soil northeast of Vohitsiombe, in the province of Fort Dauphin, Madagascar, August 3, 1926; Muséum National d'Histoire Naturelle at Paris. Unfortunately the type specimen is very badly smashed so that the exact characters of the species are difficult to ascertain. From what can be seen, however, the specimen does not seem to fit into any other known species of the genus. It is hoped that better material may soon be available.

Clerodendrum Ellioti Moldenke, sp. nov. Frutex 1 m. altus; ramis ramulisque graciliusculis minute puberulis glabrescentibus perspicue lenticellatis; sarmentis gracillimis dense puberulis; foliis oppositis; petiolis gracillimis 1–2.2 cm. longis glabris; laminis tenuiter membranaceis perfragilibus brunnescentibus ellipticis 2.5–8.5 cm. longis, 2–2.5 cm. latis integris, ad apicem breviter acuminatis, at basin plerumque acuminatis, utrinque glabris vel subglabratis, subtus punctatis; inflorescentiis axillaribus cymosis paucifloris vel 1-floris; pedunculis gracillimis minute puberulis.

Shrub to about 1 m. tall; branches and branchlets rather slender, very finely puberulous, glabrescent in age, grayish-brown, rather conspicuously lenticellate, the lenticels round and small; twigs sparse, very slender, densely puberulent; nodes not annulate; principal internodes abbreviated, 0.5–2.5 cm. long; leaf-scars large and prominent on the younger parts; leaves decussate-opposite; petioles very slender, 1–2.2 cm. long, glabrous; blades thin-membranous, very fragile, brunnescent in drying, elliptic, 2.5–8.5 cm. long, 2–2.5 cm. wide, short-acuminate at the apex, mostly acuminate at the base, entire, glabrous or subglabrous on both surfaces, more or less punctate beneath; midrib very slender, flat above, prominulous beneath; secondaries very slender, flat above, very slightly subprominulous beneath, 4–7 per side, divergent-ascending, arcuate-joined near the margins beneath; vein and veinlet reticulation mostly indiscernible on both surfaces; inflorescence axillary, cymose, few-flowered, often reduced to one flower; peduncles very slender, 12–17 mm. long, minutely puberulent; pedicels very slender, 5–15 mm. long, minutely puberulent; bracts foliaceous, 2 or 4 per cyme, or caducous, 10–15 mm. long, stipitate; bractlets linear, 5–6 mm. long; calyx thin-membranous, brunnescent, elliptic-tubular, 2.5–3 cm. long, to 14 mm. wide at the middle, glabrous, 5-plicate, its rim 5-lobed, the lobes ovate, about 7 mm. long, attenuate-acuminate at the apex; corolla infundibular, its tube about 3.5 cm. long, slightly surpassing the calyx, very sparsely scattered-pulverulent or puberulent with very minute hairs on the outside, its limb about 3 cm. wide, the lobes about 1.5 cm. long; stamens and style included in the limb; fruiting-calyx coriaceous, nigrescent, about 3 cm. long, to 1.8 cm. wide, glabrate, obscurely veined, its rim conspicuously 5-lobed; fruit brown-purple.

TYPE: *George Frances Scott Elliot 2039,* on dry rocky hills at Betsilee, near Yeanar, in the interior of Madagascar, February 13, 1890; Muséum National d'Histoire Naturelle at Paris. It was originally distributed as *Barleria prionitis* Lindl. The common name "asélo" is recorded by the collector.

CLERODENDRUM EMIRNENSE f. **dentatum** Moldenke, f. nov. Haec forma a forma typica speciei laminis foliorum crasse paucidentatis recedit.

This form differs from the typical form of the species in having its leaf-blades coarsely dentate above the middle with one or two blunt or subacute teeth on each side.

TYPE: *Georges Waterlot 486*, in the vicinity of Tananarive, Madagascar, April 1922; Muséum National d'Histoire Naturelle at Paris.

CLERODENDRUM EMIRNENSE var. **diffusum** Moldenke, var. nov. A forma typica speciei recedit cymis valde laxis diffusisque plerumque late divaricatis, pedicellis saepe usque ad 5 mm. longis, et calyce plerumque 2–3 mm. longo.

This variety differs from the typical form of the species in having its cymes much more loose and diffuse during anthesis, mostly wide-spreading, the pedicels sometimes well developed and to 5 mm. long during anthesis, and the calyx mostly 2–3 mm. long.

TYPE: *Wenzel Bojer*, somewhere in Madagascar; bears the label "Kew 11.675" in the herbarium of the Muséum National d'Histoire Naturelle at Paris.

Holmskioldia angustifolia Moldenke, sp. nov. Frutex; ramis ramulisque gracilibus sarmentosis griseis dense brunneo-puberulis glabrescentibus subteretibus; sarmentis gracillimis numerosis dense brunneo-puberulis; foliis numerosissimis confertis caducis oppositis; petiolis gracillimis 1–2 mm. longis dense puberulis; laminis subchartaceis linearibus 1–3 cm. longis, 1–3 mm. latis obtusis integris arcte revolutis, utrinque dense puberulis et nigro-punctatis; cymis 3-floris.

Shrub; branches and branchlets slender, very twiggy, gray, densely brownish-puberulent when young, glabrescent in age, not noticeably lenticellate, very obscurely tetragonal or subterete; twigs numerous, very slender, densely brownish-puberulent; nodes not annulate; principal internodes often extremely abbreviated on the twigs, 1–5 mm. long or less, more elongate (to 3.5 cm.) on the older wood and on vigorous shoots; leaf-scars very corky and elevated; leaves very numerous, crowded, dropping very quickly, decussate-opposite; petioles very slender, 1–2 mm. long, densely puberulent; blades subchartaceous, linear, uniformly dark green on both surfaces, 1–3 cm. long, 1–3 mm. wide, blunt at the apex, entire, strongly revolute-margined, densely puberulent and black-punctate on both surfaces, the slender midrib and secondaries prominent beneath; inflorescence axillary and terminal, the cymes 3-flowered; peduncles solitary in the axils, filiform, 2–6 mm. long, densely cinereous-puberulent; pedicels filiform, 3–7 mm. long, densely cinereous-puberulent; bractlets apparently none or caducous; calyx campanulate-patelliform, densely cinereous-puberulent, its tube about 2 mm. long, its lobes about 2 mm. long, unequal, acute; corolla-tube about 7 mm. long, the lobes about 5 mm. long; stamens and pistil long-exserted; fruiting-calyx very thin-membranous, star-shaped, about 2 cm. wide, puberulent and black-punctate on both surfaces, the 5 lobes very broadly elliptic, about as long as the tube, rounded to a minute cusp at the apex; fruit spheroid, about 2 mm. wide, densely white-puberulent.

TYPE: *Henri Humbert 20315*, in the vicinity of Beloha, Androy, Madagascar, February 20, 1947; Muséum National d'Histoire Naturelle at Paris.

Holmskioldia Humberti Moldenke, sp. nov. Frutex vel arbor parva; ramulis gracillimis griseis minute puberulis vel glabrescentibus; sarmentis abbreviatis densiuscule puberulis; foliis oppositis; petiolis gracilibus 7–10 mm. longis dense puberulis, pilis subadpressis antrorsis; laminis subcoriaceis late ellipticis vel breviter ovatis 3–4.3 cm. longis integris, 2.2–3.7 cm. latis, ad apicem obtusis vel rotundatis, ad basin rotundatis, supra leviter puberulis, subtus dense puberulis et resinoso-punctatis; inflorescentiis axillaribus 1-floris; pedunculis filiformibus densiuscule patenti-puberulis.

Shrub or small tree; branchlets very slender, gray, rather irregular, corky-lenticellate, not plainly tetragonal, minutely puberulent or glabrescent; twigs abbreviated, rather densely puberulent; nodes not annulate; principal internodes much abbreviated, 0.2–2 cm. long; leaves decussate-opposite; leaf-scars rather large and prominent, corky; buds densely tomentellous; petioles slender, 7–10 mm. long, densely puberulent with subappressed antrorse hairs; blades subcoriaceous, grayish-green on both surfaces, broadly elliptic or short-ovate, 3–4.3 cm. long, 2.2–3.7 cm. wide, obtuse or rounded at the apex, entire, rounded at the base, finely puberulent above (more densely so along the midrib), very densely puberulent and resinous-punctate beneath; midrib slender, mostly subimpressed above, prominent beneath; secondaries slender, 4–6 per side, arcuate-ascending, subimpressed above, prominent beneath, joined in many loops some distance from the margins; vein and veinlet reticulation very abundant, rather conspicuously subimpressed above, prominulous to the ultimate divisions beneath; inflorescence axillary, 1-flowered, subequaling or shorter than the subtending leaf; peduncles filiform, 1.5–2 cm. long, rather densely spreading-puberulent; pedicels filiform, 7–10 mm. long, densely spreading-puberulent; bractlets caducous; flowers not seen; fruiting-calyx patelliform, star-shaped, thin-textured, about 2 cm. wide, venose, puberulent on both surfaces, more densely so on the outer surface, its tube campanulate, about 6 mm. long, its 5 lobes somewhat unequal, broadly elliptic, 7–8 mm. long, obtuse at the apex; fruit oblate-spheroid, about 5 mm. wide, thin-textured, densely puberulent and more or less resinous-punctate.

TYPE: *Henri Humbert 19032*, in the tropophilous forest near Ambodimagodro, among the limestone hills and plateaus of Ankarana, Diego-Suarez, Madagascar, at an altitude of 150–200 m., December 1937 or January 1938; Muséum National d'Histoire Naturelle at Paris.

Holmskioldia madagascariensis Moldenke, sp. nov. Frutex vel arbor; ramulis graciliusculis adpresso-puberulis glabrescentibus; sarmentis gracillimis densissime breviterque sordido-pubescentibus; foliis oppositis; petiolis gracillimis 1.3–2 cm. longis dense breviterque albido-pubescentibus, pilis adpressis antrorsis; laminis firme chartaceis vel subcoriaceis, subtus albidis, lanceolato-ovatis 4–8 cm. longis, 1.8–4 cm. latis integris, ad apicem acutis, ad basin plerumque acutis saepe asymmetricis, supra densiuscule breviterque subadpresso-pubescentibus, subtus densissime breviterque pubescentibus; inflorescentiis axillaribus; pedunculis plerumque 1-floris gracillimis 1.3–2.1 cm. longis densissime breviterque pubescentibus.

Shrub or tree; branchlets irregular, dark, rather slender, not plainly tetragonal, appressed-puberulent, glabresent in age; twigs very slender, very densely short-pubescent with sordid-grayish hairs; nodes not annulate;

principal internodes 0.3–2 cm. long, mostly greatly abbreviated; leaf-scars very large and prominent, corky-margined; leaves decussate-opposite; petioles very slender, 1.3–2 cm. long, densely short-pubescent with appressed whitish antrorse hairs; blades firmly chartaceous or subcoriaceous, light green above (brunnescent in drying when immature), whitish beneath, lanceolate-ovate, 4–8 cm. long, 1.8–4 cm. wide, acute at the apex, mostly acute and occasionally asymmetric at the base, entire, rather densely short-pubescent with subappressed whitish hairs above, very densely short-pubescent (especially on the venation to its ultimate divisions) beneath, the hairs whitish and subappressed; midrib slender, subimpressed above, prominent beneath; secondaries slender, 4–6 per side, arcuate-ascending, subimpressed above, prominent beneath; vein and veinlet reticulation very abundant, rather conspicuous and subimpressed above, very prominent beneath; inflorescence axillary on the young twigs, shorter than the subtending leaf; peduncles solitary in each axil, very slender, 1.3–2.1 cm. long, very densely short-pubescent like the twigs, usually 1-flowered and with a single pair of bractlets; pedicels very slender, 5–9 mm. long, very densely short-pubescent like the twigs; bractlets linear, 3–4 mm. long, densely short-pubescent; calyx campanulate, very densely white-tomentellous, its tube about 4 mm. long and wide, its 5 lobes 3–4 mm. long, broadly ovate, acute; corolla densely white-tomentellous on the outer surface; fruiting-calyx patelliform, star-shaped, herbaceous, about 2 cm. wide, very densely short-pubescent or tomentellous on both surfaces, the lobes broadly ovate, about 6 mm. long and wide, acute.

TYPE: Service Forestier no. 50, at Ankarafansika, in the Seventh Reserve, Madagascar, before April 4, 1933; Muséum National d'Histoire Naturelle at Paris.

HOLMSKIOLDIA MICROCALYX var. **glabrescens** Moldenke, var. nov. A forma typica speciei recedit laminis foliorum chartaceis, supra leviter puberulis et venis elevatis, subtus leviter puberulis resinoso-punctatis, et calyce fructifero utrinque leviter obscureque puberulo.

This variety differs from the typical form of the species in having its leaves merely chartaceous, lightly puberulent and with elevated venation above, lightly puberulent and resinous-punctate beneath, and the fruiting-calyx merely lightly and very obscurely puberulent on both surfaces.

TYPE: Service Forestier no. 17, on sandy slopes at an altitude of 100 m. in the Seventh Reserve, Madagascar, before April 4, 1933; Muséum National d'Histoire Naturelle at Paris. The variety is said to grow to 12 m. tall, with a trunk diameter of 40 cm., blooming in November, its wood used in construction. A common name is "mafangalaty."

Holmskioldia microphylla Moldenke, sp. nov. Frutex vel arbor parva; ramis ramulisque gracillimis brunnescentibus dense adpresso-puberulis glabrescentibus saepe perspicue lenticellatis; foliis oppositis numerosis parvis; petiolis gracillimis 2–11 mm. longis dense albo-tomentellis; laminis leviter chartaceis, subtus albidis, late ovatis vel orbicularibus vel ellipticis 0.6–2.7 cm. longis, 0.5–2 cm. latis integris, ad apicem rotundatis vel emarginatis, ad basin subacutis vel rotundatis, supra dense breviterque pubescentibus vel puberulis, subtus densissime albo-tomentellis; inflorescentiis axillaribus

plerumque brachiatis interdum 1-floris; pedunculis filiformibus 7–15 mm. longis albo-pubescentibus.

Shrub or small tree, to 2 m. tall; branchlets and branches very slender, brunnescent, densely appressed-puberulent when young, glabrescent in age, very obscurely tetragonal or subterete, sometimes conspicuously lenticellate with round corky lenticels; nodes not annulate; principal internodes 0.5–2 cm. long or less; leaf-scars comparatively large, prominent, corky; buds densely tomentose; leaves decussate-opposite, abundant, small; petioles very slender, 2–11 mm. long, densely white-tomentellous; blades thin-chartaceous, gray-green above, whitish beneath, from broadly ovate or orbicular to elliptic, 0.6–2.7 cm. long, 0.5–2 cm. wide, rounded or emarginate at the apex, entire, subacute or rounded at the base, densely short-pubescent or puberulent above, very densely matted-tomentellous with white hairs beneath; midrib slender, flat or obscure above, prominulous beneath; secondaries very slender, 3 or 4 per side, arcuate-ascending, obscure or indiscernible above, prominulous beneath, they and the midrib sometimes very prominent beneath, arcuately joined some distance from the margins beneath; vein and veinlet reticulation usually indiscernible above, sometimes prominulous beneath; inflorescence axillary, usually branched and several-flowered, sometimes 1-flowered; peduncles filiform, 7–15 mm. long, densely white-pubescent with very short subappressed antrorse hairs; pedicels filiform, 4–5 mm. long, densely white-pubescent like the peduncle and its branches; bractlets linear, 1–2 mm. long, densely white-pubescent with appressed antrorse hairs; calyx patelliform, its tube about 4 mm. long, densely white-pubescent with appressed antrorse hairs, its limb 5-lobed, its lobes about 3 mm. long and 2.5 mm. wide, subacute at the apex, densely puberulent on both surfaces; corolla white or greenish, with one violet lobe or suffused throughout with violet, its tube about 6 mm. long, densely white-pubescent on the outside, its lobes unequal, 5–10 mm. long; stamens and pistil long-exserted; fruiting-calyx much enlarged, 1.2–1.9 cm. wide, very densely white-pubescent on both surfaces, the 5 lobes lingulate, 3.5–4 mm. long, usually shorter than or equaling the tube; fruit oblate-spheroid, about 4 mm. wide, densely white-pubescent.

Type: *Raymond Decary 9402*, in the upper valley of the Manambolo, southeastern Madagascar, November 24, 1931; is deposited in the herbarium of the Muséum National d'Histoire Naturelle at Paris.

Holmskioldia microphylla var. **glabrescens** Moldenke, var. nov. A forma typica speciei foliis calyceque minutissime adpresso-puberulis vel subglabrescentibus resinoso-punctatis recedit.

This variety differs from the typical form of the species in having its leaves and calyxes only very finely appressed-puberulent or subglabrescent and resinous-punctate.

Type: *Henri Humbert 20048*, in a tropophilous forest and xerophilous bush on reddish-brown sand, at an altitude of 80–150 m., in the vicinity of Manombo in the forest of Isonto, west of Ankililoaka, Madagascar, January 28, 1947; Muséum National d'Histoire Naturelle at Paris.

Holmskioldia mira Moldenke, sp. nov. Frutex vel arbor parva ca. 5 m. alta; ramis ramulisque gracilibus griseis lenticellatis glabris; foliis oppositis aromantics; petiolis gracilibus 3–12 mm. longis, supra canaliculatis pilosulo-

puberulisque, caetera glabris; laminis sub anthesin tenuissime membranaceis brunnescentibus vel nigrescentibus, in maturitate firme chartaceis et plerumque non nigrescentibus, ellipticis vel late ellipticis vel ovoideis 3–8 cm. longis, 2–5 cm. latis, ad apicem acutis vel obtusis vel rotundatis usque ad emarginatis, ad basin plerumque obtusis vel rotundatis, utrinque glabris (costa supra plerumque leviter pilosula); inflorescentiis axillaribus, 1- vel 2-floris; pedunculis filiformibus, glabris (praeter pulvinum villosum basilarem).

Shrub or small tree to 5 m. tall or more; branches and branchlets slender, gray, lenticellate, very obscurely tetragonal or subterete, glabrous; leaf-scars large and prominent, corky; lenticels often prominent and corky; buds densely tomentose-villous; nodes not annulate; principal internodes 0.2–3.5 cm. long, those on the twigs usually much abbreviated; leaves decussate-opposite, aromatic; petioles slender, 3–12 mm. long, canaliculate above and rather densely pilosulous-puberulent there, otherwise subglabrate; blades very thin-membranous and nigrescent or brunnescent during anthesis, firmly chartaceous at maturity and then usually not nigrescent, varying from elliptic or broadly elliptic to ovoid, 3–8 cm. long, 2–5 cm. wide, from acute or obtuse at the apex to rounded or emarginate, from acute to usually obtuse or rounded at the base, usually lightly pilosulous along the midrib above, otherwise glabrous on both surfaces, entire, very shiny when mature; midrib slender, flat above, prominulous beneath; secondaries slender, 5–7 per side, arcuate-divergent, mostly obscure or indiscernible at anthesis, prominulent on both surfaces when the leaves are mature, joining in many loops some distance from the margins beneath; vein and veinlet reticulation very abundant, mostly indiscernible at time of anthesis, conspicuously prominulous on both surfaces when the leaves are mature; inflorescence axillary; flowers solitary or paired in each axil; peduncles filiform, 1–3 cm. long, issuing from a villous cushion-like base, otherwise glabrous, bearing an opposite or subopposite pair of filiform bractlets 1.5–2 mm. long at its apex; pedicels filiform, 5–8 mm. long, glabrous; calyx patelliform, its tube 3–4 mm. long, its limb somewhat irregularly 5-lobed, membranous, 1.1–1.4 mm. wide, the lobes about 4 mm. long and 3.5–5 mm. wide, rounded and apiculate, glabrous on both surfaces, venose; corolla pale-blue or greenish-blue; stamens 4, long-exserted; filaments glabrous, about 2 cm. long; pistil equaling or surpassing the stamens; fruiting-calyx much enlarged, star-shaped, firmly chartaceous, very venose and shiny, 2–3.5 cm. wide, the acute or rounded lobes separate only half or 2/3 to the base, glabrous on both surfaces; fruit oblate, 3–4 mm. long, about 4 mm. wide, thin-shelled, shiny, glabrous, not plainly sulcate nor venose.

TYPE: Service Forestier no. 22, at Ankarafantsika, in the Seventh Reserve, Madagascar, before April 4, 1933; Muséum National d'Histoire Naturelle at Paris.

HOLMSKIOLDIA MIRA var. **fissa** Moldenke, var. nov. A forma typica speciei recedit laminis foliorum usque ad 8.5 cm. longis et 7.5 cm. latis, calyce fructifero 3.5–4.3 cm. lato, lobis paene ad basin fissis, fructibus 7 mm. longis latisque valde duris lignosis pernitidis brunneis sulcatis venosis.

This variety differs from the typical form of the species in having its leaf-blades to 8.5 cm. long and 7.5 cm. wide, the fruiting-calyx 3.5–4.3 cm. wide, its lobes separate almost to the base (to within 5 mm. of the base), and

the fruit when mature about 7 mm. long and wide, very hard and woody, very shiny, brown, sulcate, and venose, splitting at maturity into 4 parts.

TYPE: *Bernier 360*, at Lingvatou, Madagascar, about 1834; Muséum National d'Histoire Naturelle at Paris.

Premna aureolepidota Moldenke, sp. nov. Arbor 6 m. alta; ramulis mediocriter gracilibus obtusissime tetragonis, in juventute flavido-puberulis, in senectute cinereis, dein glabrescentibus; foliis oppositis; petiolis mediocriter crassis 1.5–2 cm. longis densissime flavido-puberulis; laminis tenuiter chartaceis vel submembranaceis, supra brunnescentibus, ellipticis 7–10 cm. longis, 3.5–5 cm. latis, ad apicem longe acuminatis integris, ad basin acutis, supra minutissime puberulis, subtus densissime puberulis et resinoso-lepidotis; inflorescentiis cymosis parvis multifloris congestis ubique dense resino so-lepidotis et sordido-puberulis.

Tree about 6 m. tall; branchlets medium-slender grayish, very obtusely tetragonal, densely flavidous-puberulent when young, more cinerous in age, finally glabrescent; nodes annulate; principal internodes 0.5–2.5 cm. long; leaf-scars subcordate, corky-margined; leaves decussate, deciduous; petioles medium stout, 1.5–2 cm. long, very densely flavidous-puberulent; leaf-blades thin-chartaceous or almost membranous, brunnescent above in drying, much lighter beneath, elliptic, 7–10 cm. long, 3.5–5 cm. wide, long-acuminate, entire, acute at the base, very minutely scattered-puberulous above, very densely resinous-lepidote on the lamina beneath and densely puberulent on the venation, the hairs sordid-incanous; midrib slender, flat above, prominent beneath; secondaries very slender, 4–6 per side, arcuate-ascending, flat above, prominulous beneath, joined in many loops near the margins beneath; vein and veinlet reticulation obscure above, prominulous beneath; inflorescence cymose, the cymes small, many-flowered, congested, densely resinous-lepidote and sordid-puberulent throughout, 1–2 cm. wide; calyx cupuliform, light-textured, herbaceous, not nigrescent, about 3 mm. long, the rim 4-toothed, the teeth ovate-triangular, about 1 mm. long and wide, acute; corolla (in bud) very small, its tube abbreviated, included, its lobes 4, about 2 mm. long and wide, rounded at the apex, cucullate, resinous-lepidote on the outside; stamens 4, included; filaments about 1 mm. long; anthers 1.5 mm. long, 2-celled, dehiscing by longitudinal slits; pistil (in bud) included; ovary subglobose, about 0.7 mm. long and wide, glabrous; style about 0.4 mm. long, glabrous; stigma bifid, its branches erect, 0.3 mm. long.

TYPE: *Jacques Leandri 587*, at Tsingy du Bemaraha, in the Ninth Reserve, Madagascar, November 23, 1932; Muséum National J'Histoire Naturelle at Paris. A vernacular name recorded by the collector is "tatamborondrao."

Premna lepidella Moldenke, sp. nov. Arbor parva; ramulis graciliusculis obtusissime tetragonis, in juventute dense cinereo-puberulis, in senectute glabrescentibus; foliis oppositis; petiolis gracillimis 1.5–3 cm. longis dense minuteque puberulis; laminis membranaceis brunnescentibus ellipticis 5.5–10.5 cm. longis, 1.5–4 cm. latis integris, ad apicem longe acuminatis, ad basin acutis, utrinque sparsissime minuteque puberulis, subtus densissime resinoso-lepidellis; inflorescentiis cymosis sparsis parvis multifloris 1.5–2 cm. latis densiusculis ubique densissime cinero-puberulis.

Small tree; branchlets rather slender, grayish, very obtusely tetragonal,

the youngest parts densely cinereous-puberulent, glabrescent in age; nodes not annulate; principal internodes very much abbreviated on young parts, to 4 cm. long on older wood; leaves decussate; petioles very slender, 1.5–3 cm. long, densely but very minutely puberulent; blades membranous, brunnescent in drying, somewhat lighter beneath, elliptic, 5.5–10.5 cm. long, 1.5–4 cm. wide, long-acuminate, entire, acute at the base, very sparsely and minutely puberulent on both surfaces, the lamina very densely resinous-lepidote with extremely minute scales beneath; midrib slender, slightly impressed above, prominent beneath; secondaries very slender, 6 or 7 per side, ascending, rather straight except for the slightly arcuate tips, not plainly joined, flat above, prominulous beneath; vein and veinlet reticulation obscure or indiscernible above, only the largest parts plainly visible beneath, the tertiaries very slightly subprominulous or flat; inflorescence cymose, sparse, small, many-flowered, 1.5–2 cm. wide, rather dense, very densely cinerous-puberulent throughout; calyx cupuliform, tightly appressed to the corolla, about 1.5 mm. long and wide, its rim truncate, very minutely 4-apiculate; corolla tubular, its tube about 5 mm. long, swollen above the calyx, densely puberulent on the outside, its lobes 5, triangular-ovate, acute, one considerably larger than the rest, puberulent on the outside; stamens 4, inserted about half-way up the corolla-tube, arched, exserted; ovary ovoid, about 1.3 mm. long and wide, glabrous; fruiting-calyx about 2 mm. long and 4 mm. wide, densely cinereous-puberulent, its rim minutely apiculate.

TYPE: *Henri Humbert 14287*, in part, on a limestone plateau in the forest of Analafanja, north of Fiherenana, at an altitude of 950–1000 m., Madagascar, March 1934; Muséum National d'Histoire Naturelle at Paris; mounted on the same sheet with a sterile specimen of something not verbenaceous.

Premna perplexans Moldenke, sp. nov. Frutex vel arbor usque ad 15 m. alta; ramulis obscure tetragonis griseis minute puberulis glabrescentibus; sarmentis densiuscule cinereo- vel flavido-puberulis; foliis oppositis brunnescentibus; petiolis gracillimis 1–2.5 cm. longis dense adpresso-puberulis, supra valde canaliculatis; laminis sub anthesin tenuiter membranaceis et valde brunnescentibus, dein tenuiter chartaceis, ellipticis vel late ellipticis vel obovato-ellipticis 4–13 cm. longis, 2–8.5 cm. latis, ad apicem acutis vel breviter acuminatis, ad basin acutis vel subacuminatis vel obtusis, integris utrinque plusminusve leviter puberulis, subtus dense resinoso-punctatis; cymis axillaribus furcatis multifloris vel subpaucifloris.

Shrub or tree to 15 m. tall; branchlets very obscurely tetragonal, grayish, minutely puberulent on the younger parts, glabrescent in age; twigs rather densely cinereous- or flavidous-puberulent with very minute hairs; nodes not annulate; principal internodes often extremely abbreviated or elongate to 5 cm.; leaves descussate, brunnescent in drying; petioles very slender, 1–2.5 cm. long, decidedly canaliculate above, densely appressed-puberulent; blades thin-membranous and very brunnescent at time of anthesis, later thin-chartaceous and not so brunnescent, from elliptic or broadly elliptic to obovate-elliptic, 4–13 cm. long, 2–8.5 cm. wide, acute or very shortly acuminate at the apex, from acute or subacuminate to obtuse at the base, entire, more or less lightly puberulent with minute hairs on both surfaces at time of anthesis, less so in age, densely resinous-punctate beneath; midrib

slender, flat above, prominent beneath; secondaries very slender, arcuate-ascending, sometimes widely divergent and then abruptly arcuate-ascending near the margins, flat above at time of anthesis but slightly prominulous at maturity, prominulous beneath; vein and veinlet reticulation obscure or indiscernible at time of anthesis, more or less prominulous on both surfaces at maturity; inflorescence axillary, cymose; cymes several times furcate but the first furcation only conspicuous, 2.5–3.5 cm. long, 1.5–3 cm. wide, many-flowered or rather few-flowered, only the ultimate portions dense; peduncles slender, flattened, 8–17 mm. long, densely appressed-puberulent, widened and 2-bracteolate at the apex; cyme-branches appressed-puberulent; bractlets linear 0.5–1.5 mm. long, densely puberulent; calyx campanulate, about 1.5 mm. long and 1 mm. wide, puberulent, its rim subtruncate, minutely 4-toothed; corolla hypocrateriform, its tube about 3 mm. long, its 4 lobes about 2 mm. long, one longer than the other 3, rounded at the apex, densely pilose-puberulent on both surfaces; stamens equaling the corolla-tube; pistil very short and included; fruiting-calyx broadly campanulate, about 2 mm. long and 4 mm. wide, its rim truncate, very minutely 4-apiculate, puberulent; fruit drupaceous, elliptic, about 5 mm. long and 3 mm. wide, glabrous, nigrescent.

TYPE: *Henri Perrier de la Bâthie 258*, among gneiss rocks in rocky woods at Antunimbary, near Mevitanunu, Madagascar, August 1898; Muséum National d'Histoire Naturelle at Paris.

Verbena goyazensis Moldenke, sp. nov. Herba ca. 1.5 m. alta; caulibus crassis obtuse tetragonis valde 4-sulcatis densiuscule scabro-pubescentibus; foliis oppositis sessilibus firme chartaceis fragilibus ellipticis 3–5 cm. longis, 1.2–2 cm. latis, ad apicem basinque acutis regulariter serratis (dentibus rotundatis revolutis), supra valde scabro-pubescentibus subbullatis (dein albo-pustulatis), subtus in venis adpresso-puberulis; inflorescentiis terminalibus paniculatis; pedunculis 4–6-aggregatis.

Herb about 1.5 m. tall; stems coarse, obtusely tetragonal, deeply 4-sulcate, rather densely scabrous-pubescent with short whitish hairs; nodes annulate; principal internodes 1.5–5 cm. long; leaves decussate, sessile; blades firmly chartaceous but fragile, grayish-green on both surfaces, elliptic, 3–5 cm. long, 1.2–2 cm. wide, acute at the apex and base, regularly serrate with rounded revolute-margined teeth, very scabrous-pubescent above (the hairs soon deciduous, leaving the lamina white-pustulate) and subbullate, appressed-pubescent on the venation beneath; midrib slender, impressed above, very prominent beneath; secondaries slender, 5–7 per side, ascending, not arcuate, impressed above, very prominent beneath; vein and veinlet reticulation very abundant, deeply impressed above and very prominent beneath; inflorescence terminal, paniculate; peduncles aggregated in groups of 4–6, slender, 2.5–34 cm. long, irregularly glandular-pubescent, its branches similar in all respects; floriferous spikes small, less than 1 cm. long, densely many-flowered, erect; bractlets lanceolate, 2.5–3 mm. long, attenuate at the apex, densely long-strigose and also more or less glandular; calyx about 1 mm. long; corolla very small, red, its tube about 1 mm. long, its limb about 1 mm. wide.

TYPE: *Goro Hashimoto 663*, at Rio de Peixa, Municipio de Goiás, Goyaz, Brazil, August 7, 1949; Instituto de Botânica at São Paulo.

VERBENA OCCULTA f. **alba** Moldenke, f. nov. A forma typica speciei corollis albis recedit.

This form differs from the typical form of the species in having white corollas.

TYPE: *Ramón Ferreyra 6586*, in a grassy steppe, at an altitude of 3800–3900 m., between San Rafael and Cerro de Pasco, dept., Pasco, Peru, January 31, 1950; Britton Herbarium, the New York Botanical Garden.

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TORREYA

William J. Bonisteel. 1892–1949.*

William J. Bonisteel was born in Port Huron, Michigan, November 30, 1892 and died in Mexico City, December 12th, 1949. His education included Ph.C. University of Michigan 1918; M.A., Ph.D. Columbia University, 1926, 1939. He served on the faculty of Columbia College of Pharmacy, 1920–1925 and on the faculty of Fordham College of Pharmacy and the Graduate School, 1925–1942. During the period 1940–1942 he was editor of TORREYA. In war service, he became Head Drug Specialist, Office of Coordinator of Inter-American Affairs, and was on the Board of Economic Warfare 1942–.

Professor Bonisteel was a man of great energy and enthusiasm. He was outstanding at Fordham for generosity, cooperation and loyalty. Pharmacist, botanist and geneticist, he was interested in the breeding of better drug plants and maintained a considerable collection of species of *Aconitum*, at his farm in Pine Plains, N. Y. He also worked with *Cinchona*, *Digitalis* and *Hydrastis*. He was active in all phases of the Torrey Club's activities and led overnight field trips to the Beaverkill and Doubletop Mountain. In addition to teaching, writing and his own research, he found time to direct the research of graduate students in plant breeding and cytology. Ever friendly and willing to assist, he will be remembered by his colleagues and students.—C. A. BERGER, Fordham University.

BOOK REVIEWS

Drawings of British Plants. Part III. Cruciferae by S. Ross-Craig. Foreword by E. Salisbury. 77 plates. G. Bell & Sons, London. 1949. 9s. net.

* Photograph printed with the assistance of the Biological Laboratory, Fordham University.

This is the third part of a series of illustrations of plants of the British Isles. The first deals with the Ranunculaceae, the second with the Berberidaceae, Nymphaeaceae, Papaveraceae and Fumariaceae, the third with the Cruciferae. Ultimately, we are told, there will be 1500 to 1800 plates, of which 77 are included in this part. The aim is to "illustrate all the clearly defined species native to the British Isles," excepting those found only in the Channel Islands and some doubtful species and microspecies. "Aliens are included if they have become established, or are becoming established over a wide area."

One plate is devoted to each species, and it normally includes a life-size illustration of the plant or of a portion of the plant, as well as enlargements of flowers, flower parts, fruits, seeds, and other characteristic features. Underground portions of the plant are usually shown. These drawings are three-dimensional, clear, and forcefully and effectively done. Latin names, common names, flower color, and sometimes brief notes are given with each plate. There are no floral diagrams, and there is no text, though the volume is provided with a foreword, an introduction and an index. It should be of special value to those who are interested in the external morphology and identification of flowering plants.

The work has the blessing of the Director of the Royal Botanic Gardens at Kew, who, in his foreword, rightly commends it for its "combination of artistic portraiture and scientific accuracy of detail."—EDWIN B. MATZKE.

Webs in the Wind. By Winifred Duncan. The Ronald Press Company. New York 10, N. Y. $4.50

This latest addition to the Humanizing Science Series is also a contribution to English literature. Written in a charming style and attractively illustrated, this book will delight every lover of nature. Even as her spiders weave their webs, the authoress, with the touch of Henri Fabre, spins a tale of science that fascinates and holds one enmeshed in its beauty. We feel the rain drops of the sudden squall and run to shelter across the sandy barren. We probe the darkness of the shed with our flashlight and come face to face with the spider—and the tale unfolds.—E. RUTH WITKUS.

Shrubs of Michigan. By Cecil Billington. Cranbrook Institute of Science, Bloomfield Hills, Michigan. $4.50.

A second edition of a very successful popular treatise. It is well printed and the illustrations are excellent. The keys, pictorial glossary and the clearly written descriptions simplify identification. Two hundred and seven species are described.—C. A. BERGER.

PROCEEDINGS

Minutes of the Meeting of February 7, 1950. The meeting was called to order at Columbia University at 8:00 p.m. by President Clum. Thirty-nine members and friends were present. Dr. George L. McNew of the Boyce Thompson Institute for Plant Research spoke on "The Natural and Synthetic Quinones in Relation to Plant Functions."

After discussion the meeting was adjourned at 9:10 p.m.

Minutes of the Meeting of February 21, 1950. The meeting, at Hunter College, was opened at 8:25 p.m. by President Clum; 28 members and friends were present. The minutes of the preceding meeting were read and approved. Leopold A. Charette, Burlington, Vermont; Read C. Rollins, Cambridge, Mass.; and John H. Thomas, Stanford, California were proposed for active membership; all were elected. A number of resignations were accepted with regret. Announcement was made of the election by the Council of Miss Honor Hollinghurst as member of the Council for 1950–51, and Dr. M. A. Johnson for 1950, to serve in place of Dr. Hitchcock and Dr. Black, recently elected vice-presidents. Mr. John E. Cantlon of Rutgers University presented a discussion

of "Some Responses of Vegetation to Microclimate in Central New Jersey." His abstract follows:

A study of the microclimates of steep, vegetated north- and south-facing slopes has revealed striking differences between the slopes in maximum air temperature, mean air temperature and vapor-pressure deficit increase, markedly nearer the surface. In addition to these differences in microclimate between the slopes, there are marked differences in length of growing season, duration of snow cover, severity of summer drought, etc.

Quantitative studies of the composition and structure of the vegetation on the two slope exposures reveal marked differences between the slopes. The magnitude of the difference between the slopes increases near the surface, the difference between the terrestrial bryophyte layers being greatest. This seems to suggest that while all plants must spend part of their existence in the lower layers of the atmosphere, those which do not rise above this level are most effected by it.

The meeting was adjourned at 9:40; refreshments were served by the botany staff of Hunter College.

Respectfully submitted,
DONALD P. ROGERS,
Recording Secretary

FIELD TRIP REPORTS

May 6. West Park, N. Y. The group visited Slabsides, the rustic cabin where John Burroughs did much of his writing, and on nearby walks found *Dicentra cucullaria, Trillium erectum, Erythronium americanum, Corydalis flavula, Micranthes virginiensis, Ranunculus abortivus, Arabis lyrata,* and six species of violets out in fine shape, also still some bloodroot and hepatica. Attendance 8. Leader, H. N. Moldenke.

May 7, Netcong, N. J. We had splendid weather for this trip. Twenty-one kinds of birds were reported and the usual spring flowers. Attendance 9. Leader, Major L. F. Barry.

May 13–14. Lake Shehawken, Wayne Co., Pa. Date was changed to enable the group to see *Viola Selkirkii* and several other uncommon plants in flower. Lake Shehawken is about 1800 feet above sea level and the flora in the vicinity has many northern components, not generally encountered in the Torrey range. *Viola incognita, V. labradorica* and eight other violets were seen in flower. Other interesting species were *Potentilla procumbens* (only station in the U.S.A. though it occurs in Labrador, Newfoundland, etc.), *Lonicera canadensis, Hepatica acutiloba, Claytonia caroliniana, Luzula saltuensis, Lycopodium tristachyum, L. annotinum, L. clavatum, Polystichum Braunii, Dryopteris Goldiana, Botrychium obliquum* var. *oneidense,* and *Cetraria islandica* (one of four known stations for Iceland moss in Penna.). The success of the trip was further assured by cool, clear weather and the hospitality of Mr. Dix's brother whose idea of practical botany took the form of "stacks" of delicious pancakes with plenty of home made maple syrup. Any calls for a repeat on this trip? Attendance 7. Reported by Louis E. Hand.

May 19–21. Branchville (N. J.) Nature Conference. The Pines having been rechristened and converted into a short season cocktail resort, we were happy to return to the excellent meals and good service of the Haltere Hotel on Culvers Lake. Jim Hawley and Wallace Husk put on their usual excellent program and we are also grateful to the leaders and helpers who served with them to make our 29th annual nature conference a success. The evening programs included an extraordinary marine life colored motion picture with a lecture by Mr. Frank Trenor, beautiful bird films by the Schaughencys, and star gazing with the Roths. Daily bird walks, at all hours, were led by Dave Fables, Mr. and Mrs. James W. Hand and the Schaughencys. At the Sunday summary it was found that these industrious people had turned in an all time high bird list of 121 species, including 28 different warblers. Plant walks were conducted by Lou Hand around the Haltere vicinity and that of the former Hotel Pines. Seventeen species of plants were

added to the list compiled by Harold Moldenke in 1948, bringing the total to 639 species. Attendance was about 100. Reported by L. E. Hand.

May 21. Arden, N. Y. The group split up into three sub-groups as follows: (1) Those interested particularly in mosses visited the Surebridge Swamp. (2) Some, more interested in walking, followed the Lichen Trail for a way. (3) Others spent the day examining lichens in the area of Island Pond. The weather was fine. Attendance 13. Leader, Albert Schatz.

May 27. High Point State Park, N. J. Participants in the circuit of the Rhododendron swamp observed the very interesting proximity of such species as pitch and white pines, black spruce, hemlock and southern white cedar, a not too common blending of northern and southern elements. The season was approximately two weeks behind schedule and the wild calla was not in bloom, though most other seasonal species were. The painted trillium was the most attractive species seen. Mr. Edwards located the northern jack-in-the-pulpit, (*Arisaeme stewardsonii*), a species new to most present. A lovely stand of fringed polygala was found. Very few flowers of the clintonia were in bloom. In the afternoon a visit to a new beaver dam and swamp was enjoyed. The water table had been elevated some six inches, the effects of which are to be watched with interest. Already amphibians have taken advantage of the change and are abundant (mostly green frogs). The beautiful song of the northern water thrush was repeatedly heard in the Rhododendron swamp. Additional species flowering at the time were: three-leaved false solomon's seal, canada mayflower, indian cucumber, wild ginger, pink lady's slipper, rue and wood anemone, small-flowered crowfoot, swamp buttercup, wild columbine, white baneberry, sweet white and downy stemmed yellow violets, dwarf dogwood, golden ragwort. A list of plants of this area is being prepared. Leader, David Fables.

May 28. Springdale, N. J. A very interesting trip was made to the Springdale swamp. This is an especially good location for *Trollius*. Attendance 16. Leader, H. N. Moldenke.

June 4, Newburgh, N. Y. A field trip of especial interest was arranged at the home of Senator Thomas C. Desmond whose private aboretum contains 689 species of trees, shrubs and woody vines. Senator Desmond has long been a member of the Torrey Botanical Club and extended his invitation to the Field Committee on the occasion of our Christmas lunch. Forty-four members and friends arrived by chartered bus with Mr. Frazee and were joined in Newburgh by many others who had travelled by car. Though there was heavy rain in the early morning, the weather was ideal by the time we arrived.

The specimens are clearly labelled with permanent metal labels giving both scientific and common names. Much trouble has been taken to collect native woody plants from all parts of the United States and to establish them in surroundings best suited to their growth. There are also many exotic species in the collection, such as the fine specimens of *Cercidiphyllum japonicum*, *Davidia involucrata*, *Pseudolarix*, *Larix leptolepis*, *Cedrus atlantica* and its variety *glauca*, *Abies Koreana* with numerous young, erect cones, *Sciadopitys*, *Cryptomeria* and many others. Of especial interest are two fine specimens of *Torreya nucifera*, the Japanese Torreya. Senator Desmond would like to attempt to establish a tree of *Torreya taxifolia* but has not yet succeeded in obtaining one.

Flowering shrubs were abundant, many of them at the peak of their bloom. Among these were a beautiful "Golden Chain" *Laburnum*, *Neillia*, *Calycanthus*, *Cytisus*, *Chionanthus*, *Enkianthus* and others. A magnificent specimen of *Magnolia acuminata* is very tall and old. Several of the umbrella trees, *Magnolia tripetala* were in bloom.

Senator and Mrs. Desmond were generous in their hospitality. The whole house was thrown open to the guests, that we might enjoy the fine collection of antique furniture and other treasures. Mrs. Desmond is an artist and writer and we visited her attractive studio on the third floor. At one o'clock, a delicious and satisfying lunch was served on the terrace beside the house, whence we looked down upon the Hudson and towards the highlands on either side. After lunch, Senator Desmond showed us his rarest trees.

Close by the house is a very old *Tsuga Sargentii*, a densely compact weeping hemlock, its original ancestor discovered not far distant. On a more sheltered hillside is a very well established *Sequoia gigantea* and near it, a fairly large *Rhamnus purshiana*. Young and well protected are small specimens of the western pines, *Pinus aristata*, *P. contorta* and others.

We had not seen quite all of the noteworthy plants when it was time to leave, nor had we all seen the same ones. It was agreed, however, that the trip had been a treat in every way, thanks to the thoughtful planning of our host and Mr. Frazee. Reported by J. L. S. Simpson.

June 11. Oswego River, N. J. A perfect day for canoeing. We improved last years performance; it was only an hour late when we started. The Oswego River certainly shows all aspects of canoeing. At one place there are even rocks, queer in South Jersey, where a portage must be made. This year it seems to be popular to blame the season; it was so in our case. The flowers that we should have seen, with the exception of laurel, were still in bud. The laurel was magnificent. We did not find a single orchid. The Itea so conspicuous last year was not in bloom. There were no upsets. Attendance 11. Leader, Miss M. Williams.

June 25. Kittatinny Ridge, N. J. Three veteran helpers in maintaining our section of the Appalachian Trail and one new member of the Club were waiting in Blairstown for the leader and three members of the trail patrol of the New York-New Jersey Trail Conference. We started at Flatbrookville road and worked south since the northern end was most in need of attention. A very rugged type of weeder was found most effective in removing blueberry, young azalea and even light oak growth along the trail. Hedge shears, machete, and various sizes of pruning tools were also employed. A double bitted axe found use on a few down logs across the trail. Blazes were repainted.

In addition to the labor we found time to observe some plants. Several specimens of *Pogonia verticillata* were seen but none were in flower. The more broadly ovate leaves, fleshier stem, and absence of tuberous roots were noted as distinguishing this plant from the Indian cucumber-root, which was equally common. Mountain laurel was at the height of its beauty, sheep laurel was common but less showy. The pale corydalis and goat's rue made more modest floral contribution. Over a dozen different grasses and sedges were observed. Blueberries were ripening. Rock fern, ebony spleenwort, and evergreen woodfern were common. The attractive little scarlet cup fungus was unusually abundant while nice specimens were seen of the flat-topped Colybia and the woods agaric. Leader, John A. Small.

NOTES

Sigma Delta Epsilon, Graduate Women's Scientific Fraternity, takes pleasure in announcing a second research award of $500.00 to be given to a member of the organization submitting the best paper describing original research. Also a new award of $200.00 is available for the best paper describing original research carried out in the home. Both of these awards will be presented at the Sigma Delta Epsilon luncheon for All Women in Science to be held during the A.A.A.S. meeting in December 1950. Dr. Lela V. Barton, Boyce Thompson Institute for Plant Research, Yonkers, N. Y. is chairman of the Research Awards Committee.—L. V. B.

The first agricultural experiment station in America was that of Connecticut, established in 1875 under the inspired leadership of Samuel W. Johnson, professor of agricultural chemistry at Yale University. The professed purpose was that of "putting science to work for agriculture." That purpose has been well served and achievement continues. The seventy-fifth anniversary will be observed on Sept. 28 and 29. An attractive and informative leaflet and a complete program are available. There will be displays of historical and current research and plenty of speeches. The Torrey Botanical Club, oldest botanical society in America, and the BULLETIN, oldest botanical journal in America, extend congratulations to their contemporary, the **Connecticut Agricultural Experiment Station**, P. O. Box 1106, New Haven 4, Connecticut.—J. A. S.

A most interesting book has recently been issued by the Conservation Committee of the Garden Club of America, 15 E. 58th St., New York 22. Spiral bound and paper covered, it consists of 88 questions selected from thousands solicited from groups and individuals and answered by experts, sometimes by several experts. The American Museum of Natural History and its staff are prominent cooperators as well as men from the outstanding public and private institutions of similar kind throughout the country. The title is **Conservation Please!** Read it and see that your library gives it publicity.—J. A. S.

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COMPILED BY

LAZELLA SCHWARTEN

WITH THE COLLABORATION OF THE EDITOR OF THE TAXONOMIC INDEX

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